

30 November 2012 | \$10

Science



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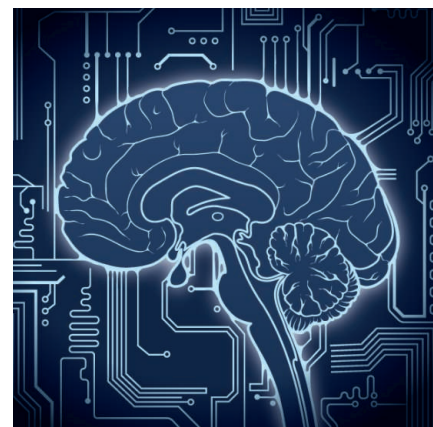
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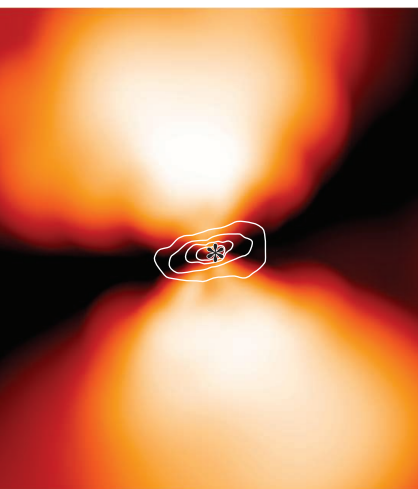
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Computer-generated models of three-dimensional nanostructures that were self-assembled from synthetic DNA strands called DNA bricks. A master collection defines a 1000-voxel "molecular canvas" with a 25-nanometer edge. By selecting subsets of bricks, Ke *et al.* constructed a panel of 102 distinct shapes with sophisticated surface features and intricate interior cavities and tunnels. These nanostructures may find applications ranging from biomedicine to nanoelectronics. See page 1177.

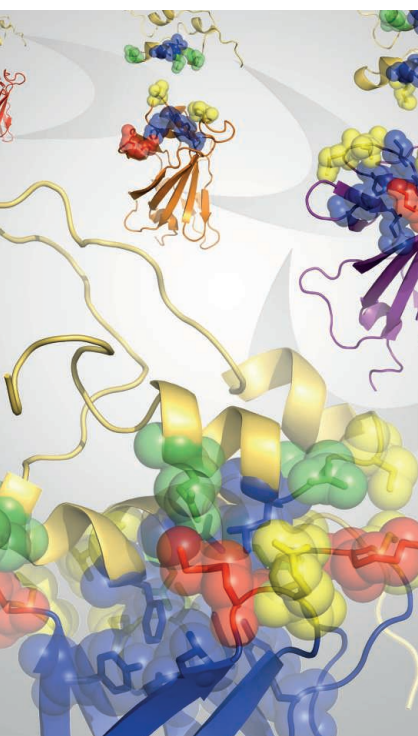
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Publication Ahead of Print

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10.1126/science.1232468

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10.1126/science.1229764

Evidence for Water Ice Near Mercury's North Pole from MESSENGER Neutron Spectrometer Measurements

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10.1126/science.1229953

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10.1126/science.1231106

Apatite ⁴He/³He and (U-Th)/He Evidence for an Ancient Grand Canyon

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10.1126/science.1229390

Crocodile Head Scales Are Not Developmental Units But Emerge from Physical Cracking

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10.1126/science.1226265

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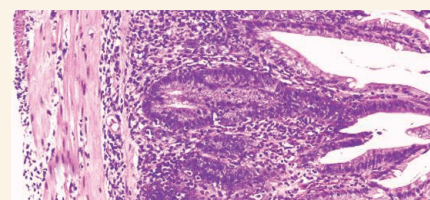
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Intestinal colitis.

SCIENCE CAREERS

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Time Off for Dad

S. Gaidos

Paternity leave helps fathers and mothers advance their careers; too bad it is not more common.

Robots, Fish, and Undergrads

S. Webb

John Long and his Vassar undergraduates study fish biomechanics and behavior.

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ADVANCING SCIENCE. SERVING SOCIETY

Warming and Melting >>



Mass loss from the ice sheets of Greenland and Antarctica account for a large fraction of global sea-level rise. Part of this loss is because of the effects of warmer air temperatures, and another because of the rising ocean temperatures to which they are being exposed. **Joughin *et al.*** (p. 1172) review how ocean-ice interactions are impacting ice sheets and discuss the possible ways that exposure of floating ice shelves and grounded ice margins are subject to the influences of warming ocean currents. Estimates of the mass balance of the ice sheets of Greenland and Antarctica have differed greatly—in some cases, not even agreeing about whether there is a net loss or a net gain—making it more difficult to project accurately future sea-level change. **Shepherd *et al.*** (p. 1183) combined data sets produced by satellite altimetry, interferometry, and gravimetry to construct a more robust ice-sheet mass balance for the period between 1992 and 2011. All major regions of the two ice sheets appear to be losing mass, except for East Antarctica. All told, mass loss from the polar ice sheets is contributing about 0.6 millimeters per year (roughly 20% of the total) to the current rate of global sea-level rise.

Building with DNA

One route for assembling three-dimensional (3D) DNA nanostructures is to start with a long natural DNA single strand and attach short strands, or “staples,” that cause the entire “origami” structure to fold into a desired shape. **Ke *et al.*** (p. 1177, see the cover; see the Perspective by **Gothelf**) present an alternative approach to 3D assembly that builds upon modular assembly of 2D DNA tiles. One hundred and two distinct shapes were created from four-domain, 32-nucleotide single-stranded DNAs that assembled like children’s blocks; each block could bind to four neighboring blocks through specific pairing interactions. Computer design and stepwise assembly allowed assembly of hollow shapes with a variety of internal cavities.

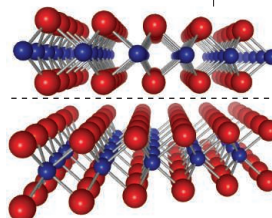
Glow from the Past

Extragalactic background light (EBL) is the integrated radiation from all extragalactic sources in the universe. Foreground emission from our solar system and galaxy makes direct detection of

the EBL very difficult. However, it is possible to measure EBL from gamma-ray spectra of distant sources, because gamma-ray photons from these sources interact with the EBL. **Ackermann *et al.*** (p. 1190, published online 1 November; see the Perspective by **Bromm**) report a measurement of the EBL based on an attenuation feature seen in the combined spectra of distant active galaxies detected by the Fermi Gamma-Ray Space Telescope. The result puts constraints on the cosmic history of star formation.

What Do You Know? A Dome

The superconducting dome—the appearance of a maximum in the transition temperature as a function of a tuning parameter—has been observed in compounds such as cuprates, pnictides, and heavy fermion materials and is thought of as a signature of unconventional superconductivity. **Ye *et al.*** (p. 1193) used a liquid gating technique



combined with back gating to finely tune the carrier density in the band insulator MoS_2 , which allowed them to observe the formation of a dome. The unexpected finding awaits theoretical explanation but may suggest that the appearance of an optimal carrier density may be a more common occurrence than was previously thought.

Forming the Regular Moons

There is a good understanding of how Jupiter’s Galilean moons (Io, Europa, Ganymede, and Callisto) and Saturn’s largest moon, Titan, formed, but the same is not true for the other regular moons of the giant planets. **Crida and Charnoz** (p. 1196) describe a model whereby most of the regular moons (those with little orbital inclination or eccentricity) in the solar system were born in ancient massive rings that surrounded their planets. This model does not apply to Jupiter, but it can explain the regular moons of the other giant planets, including Titan, and even those of Earth and Pluto. The model further predicts that Uranus and Neptune once had massive, Saturn-like rings, which gave birth to moons and then disappeared.

Swimming in Iron Pools

Because iron is essential for marine phytoplankton growth, its availability limits the primary productivity of the oceans. Iron is typically bioavailable only when present in a dissolved state; however, a large fraction of the total iron in the oceans exists as tiny solid-phase particles ranging in size from a few nanometers to a few micrometers. **von der Heyden *et al.*** (p. 1199) used high-resolution x-ray microscopy and spectroscopy to characterize the distribution of iron particles along two transects of the Southern Ocean. Analysis of a number of individual particles reveals strong variation in iron oxidation state, particle mineralogy, and substitution of aluminum for iron—all of which control the solubility, and hence bioavailability, of iron.

Modeling the Brain

Neurons are pretty complicated cells. They display an endless variety of shapes that sprout highly variable numbers of axons and dendrites; they sport time- and voltage-dependent ion channels along with an impressive array of neurotransmitter receptors; and they connect intimately with near neighbors as well as former neighbors who have since moved away. Simulating a sizeable chunk of brain tissue

has recently become achievable, thanks to advances in computer hardware and software. **Eliasmith et al.** (p. 1202; see the Perspective by **Machens**) present their million-neuron model of the brain and show that it can recognize numerals, remember lists of digits, and write down those lists—tasks that seem effortless for a human but that encompass the triad of perception, cognition, and behavior.

More Genes, Tougher Plant

Soybean crops, which supply valuable protein, oil, and renewable fuel, are under attack by a nematode for which there is no effective pesticide. Instead, agriculture relies on resistance derived from a genetic locus, which is now represented in most of the soybean crops cultivated in the United States. **Cook et al.** (p. 1206, published online 11 October) elucidated the mechanisms by which this *rhg1-b* allele protects against disease. The region carries several genes, none of which resemble other known immune receptor genes. Experiments silencing one or another of the genes showed that the genes work as a cluster. However, one set of the genes is not enough: Plants need multiple repeats of the locus to acquire resistance.

Discerning a Difference

Neighboring cells communicate via the Notch signaling pathway to make numerous decisions. Notch receptors are known to distinguish between two distinct ligand families, Delta and Serrate/jagged, in different contexts. Posttranslational sugar modifications have been shown to play a role in this process, but it is not clear if other features of Notch are involved. Using a forward genetic approach in fruit flies, **Yamamoto et al.** (p. 1229) identified an evolutionarily conserved amino acid in the extracellular domain of Notch necessary for Serrate/jagged signaling but dispensable for Delta signaling.



DNA Repair in Vitro

Accurate replication of the genome is critical to an organism's continued survival. Damaged DNA not repaired before the commencement of replication can cause the DNA replication fork to stall or collapse, which can result in mutation or recombination, with potentially serious consequences for cell and organism. Fork regression involving a so-called "chicken foot" structure (Holliday junction) is thought to provide one mechanism for dealing with unrepaired DNA damage during replication. **Manosas et al.** (p. 1217) analyzed the action of the T4 bacteriophage replisome and helicase UvsW on a stalled-fork mimic in vitro, using a magnetic trap. UvsW was able to switch migration directions, which was essential for remodeling the stalled fork. Together, UvsW and T4 holoenzyme were able to drive template switching and lesion bypass in vitro.

Cooperation Is the Key to Control

Chronic infections like hepatitis C virus (HCV) or HIV are hard on the immune system. In the face of a constant threat, some immune cells like T lymphocytes become "exhausted"; although present, they can no longer mount responses that are effective enough to eliminate the virus. These responses, however, are still important because in many cases they do keep the virus relatively controlled. The mechanisms underlying the population dynamics of T cell responses during chronic viral infection, however, are not well understood. **Paley et al.** (p. 1220) now demonstrate that the T-box transcription factors T-bet and Eomesodermin differentially regulate two phenotypically and functionally distinct subsets of antiviral CD8⁺ T cells in mice. The cooperation of these subsets may be important for antiviral immunity during chronic viral infections in humans.

Joy or Pain?

Face recognition and processing are so completely central to human social interactions that these functions are supported by specialized regions in the brain. One of the fundamental aspects being processed is emotion, particularly whether the emotion being expressed is positive or negative. Nevertheless, neuroimaging studies have documented that perceiving opposite emotions often activates the same or overlapping regions. **Aviezer et al.** (p. 1225) report that the recognition of positive versus negative emotions actually relies on information communicated by the body—the extent to which perceivers identified joy versus grief in composite figures was driven by whether the body came from a joyous (versus grievous) image rather than the face.

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Growing Latin American Science

HOW CAN A COUNTRY RAPIDLY IMPROVE ITS CAPACITY IN SCIENCE, TECHNOLOGY, AND INNOVATION? Invest in people, as Latin America is doing. For Brazil, supporting this effort through international exchange and increased mobility over the past year will substantially enlarge the nation's corpus of highly qualified scientists and technologists. Argentina has increased its number of doctoral fellowships and is concentrating on recruiting back Argentine investigators working abroad to advance its own basic research and industry. And in Chile, special programs have been funding fellowships to send students abroad to obtain Ph.D. degrees in science. These activities reflect the growing desire of Latin American nations to train an educated workforce in the world's best institutions and foster "globalized" science on the continent.

Nations understand that they must equip themselves with future leaders of scientific and technological research and innovation to compete economically on the international stage. In July 2011, the Brazilian government dramatically addressed this challenge by launching the *Ciência sem Fronteiras* (CsF) program, which offers, through 2015, 100,000 scholarships for scientific study and research work abroad. The program is jointly administered by federal funding agencies and is partly funded by the private sector. This Brazilian *Science Without Borders* program has attracted the interest of universities across the globe that are eager to receive well-funded undergraduate, graduate, and postgraduate students and thereby establish collaborations with a rapidly developing economy. CsF specifically focuses on science, technology, engineering, and mathematics, and funds travel and installation expenses, health insurance, and salary for fellowships ranging from 1 to 4 years in academic or industrial centers. The program also provides incentives—start-up funds and fellowships—for out-of-country scientists to move to Brazil, whether in the academic, governmental, or industrial sector, even for brief 2-month periods.

By the end of 2012, Brazil will have selected more than 20,000 students and postdoctoral fellows to go abroad to 30 different nations in Europe, Asia, Australia, Africa, Central America, and North America.* And 250 have come to Brazil thus far through the special visiting scientist program. The national and international companies that joined the program are already competing to offer jobs to the Brazilian students as they return. As a result, Brazilian universities are increasingly engaging in international collaborations, with an unprecedented exposure already reflected in international university rankings. In the next few years, approximately 3000 Chilean students are expected to return to Chile with doctoral degrees. The challenge will be to allocate funding to support their continued training.

Latin American countries are also increasing the number of fellowships to attract talented students from developing countries, including those in Africa and Asia. For Brazil, this is being done in association with the Brazilian foreign ministry and international organizations such as The World Academy of Sciences (TWAS) and the United Nations Educational, Scientific and Cultural Organization (UNESCO).

Within Latin America, the South American Support Program of Science and Technology (PROSUL) is supporting all South American countries, with funding for joint research projects and for scientific events and research networking. Other successful programs include the Iberoamerican Program of Science and Technology (CYTED) and the African Thematic Program of Cooperation in Science and Technology (PROAFRICA).

Latin America must continue to strengthen the internationalization of its science, as well as exploit its local excellence through intracontinental collaborations, positioning the continent to become a global leader in science, technology, and innovation. Indeed, every nation can benefit by growing a capable and knowledgeable workforce.

— Celia R. S. Garcia, Armando J. Parodi, Glaucius Oliva

10.1126/science.1232223

*www.cienciasemfronteiras.gov.br/web/csf/bolsistas-pelo-mundo



EDITED BY STELLA HURTLEY AND JAKE YESTON

GEOLOGY

Toba Timing

Toba in Sumatra is thought to have been the largest volcanic eruption in the past tens of millions of years—larger than the main eruption at Yellowstone—and it formed a huge lake about 30 km wide and 100 km long. Signals of the eruption are thought to be preserved in both Greenland and Antarctic ice cores. The event has been proposed to have led to a population bottleneck in early *Homo sapiens* that is recorded in our genetic history. The exact age of the eruption, however, has been uncertain, complicating efforts at global correlation of climatic and environmental events. Storey *et al.* have now obtained a precise $^{40}\text{Ar}/^{39}\text{Ar}$ radiometric age on crystals from Toba deposits in Malaysia. The age, supported by astronomical calibrations, is 73.88 ± 0.32 thousand years ago, close to the presumed age of about 74 ka. The date helps calibrate the signals and thus correlates the age of ice cores in separate hemispheres, and the eruption timing just precedes an abrupt cooling of about 10°C recorded in the Greenland ice. — BH

Proc. Natl. Acad. Sci. U.S.A. 10.1073/pnas.1208178109 (2012).

CELL BIOLOGY

STIM1-ulating Phagocytosis

Whether the endoplasmic reticulum (ER) fuses with phagosomes has been hotly debated. What is not in dispute is the juxtaposition of these organelles within cells. However, the mechanism and functional significance of this juxtaposition remain unclear. Nunes *et al.* found that ER membranes do not fuse with phagosomes but are recruited for signaling purposes by STIM1, which regulates the store-operated calcium entry pathway. A combination of fluorescence and electron microscopy in neutrophils from STIM1-deficient mice and in phagocytic fibroblasts lacking STIM1 revealed that STIM1 recruitment to phagosomes is required for efficient phagocytosis. STIM1 also increased the juxtaposition of thin ER cisternae with phagosomes, promoted the shedding of periphagosomal actin rings, and promoted localized calcium elevations. Furthermore, the effects of STIM1 on phagocytosis, actin shedding, and

periphagosomal calcium elevations, but not on ER recruitment, required STIM1 to activate its target channels on phagosomes. Thus, STIM1-mediated ER recruitment to phagosomes, rather than initiating fusion, appears to open phagosomal calcium channels to generate localized calcium elevations that promote phagocytosis. — SMH

Curr. Biol. 22, 1990 (2012).

PHYSICS

Don't FRET—All Under Control

In photosensitive biological or chemical processes involving energy harvesting, such as photosynthesis, photons are absorbed by one molecule and the associated energy is transferred to another (or series of others) through a resonant process called Förster resonance energy transfer, or FRET. With the development of organic electronics, debate has surrounded the question of whether such systems can be designed to control the energy transfer process. Blum *et al.* conducted a systematic study of the FRET process involving two molecules (an acceptor and donor) bridged by a DNA chain that precisely established the separation distance. They altered the local optical environment by placing the molecules in the vicinity of a mirror and controlling that separation. Their main finding is that although the rate of energy transfer between the molecules is independent of what is done to the optical environment, the efficiency of that energy transfer process is sensitive. Such nanophotonic control might find

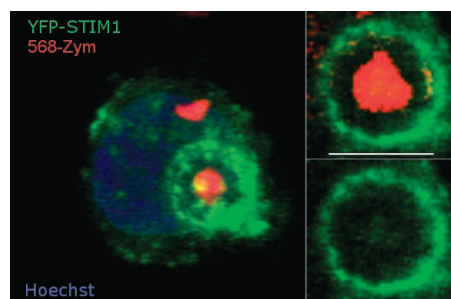
practical applications in designing better artificial molecular-based electronic energy-harvesting or lighting devices. — ISO

Phys. Rev. Lett. 109, 203601 (2012).

CHEMISTRY

Better Living Through Chloride

Conjugated polymers are used as the active element in many flexible optoelectronics devices, including thin-film transistors and light-emitting diodes, and as such, versatile routes to their preparation are in demand. Bonillo and Swager report a distinct method for the synthesis of conjugated polymers with thiophene backbones, a class of materials currently accessed through oxidative and cross-coupling techniques. Their starting point was the previously reported observation that 3-alkoxy-2-bromothiophenes undergo a violent autopolymerization that proceeds through a cationic mechanism with the elimination of HBr. The authors show that the chloro variant of this reaction proceeds in a more controlled manner. They used chlorodibutylpropylenedioxythiophene as a model monomer and studied the promotion of the reaction by different Lewis acids and solvents: Tin tetrachloride in *o*-dichlorobenzene proved the optimal combination. End-capping by nucleophiles could stop the reaction on account of the chain-growth nature of the mechanism; moreover, the “living” character of the process not only led to products with a low polydispersity index but also enabled the synthesis of block copolymers. In this



vein, the authors prepared rod-coil copolymers comprising thiophene and ethyl vinyl ether blocks. They further suggest that the ionic addition/elimination scheme could generalize, offering access to a range of other polyaromatic systems. — PDS

J. Am. Chem. Soc. **134**, 10.1021/ja308498h (2012).

ECOLOGY

Never Too Young to Learn

Recognition between parent and offspring is an essential component of reproduction for many species. Its importance is more profound for species that undergo prolonged separations followed by reunions, often facilitated through call recognition by both mothers and offspring, or for species in which there may be competition for parental attention from freeloaders. In most cases, mother and infant imprint on each others' calls shortly after birth or appear to have a genetically based recognition template. Colombelli-Négrel *et al.* show that superb fairy wrens go one step further by singing a specific incubation song to their in-egg embryos, which helps them to oust parasitizing cuckoo chicks



that have not learned the brood's "password." To ensure that both parents are in the know, females also incorporated their incubation song into begging calls given to their male partners, resulting in males also being more parental to chicks singing the right song. It is not clear why the cuckoo chicks, which are also exposed to the incubation song as eggs, do not produce accurate passwords. Perhaps the early learning ability of the wrens has allowed them to gain ground in the parasitic arms race. — SNV

Curr. Biol. **22**, 10.1016/j.cub.2012.09.025 (2012).

MICROBIOLOGY

Community Matters

We often think of bacteria as quintessentially single-celled organisms, finely tuned to survive as rugged individualists. For many prokaryotic species, this would be a mistaken impression. Quorum sensing (QS), a system of communication between cells related to population density, regulates a number of coordinated activities, including biofilm formation, swarming motility, and bioluminescence. Now Goo *et al.* show that, unlike the wild type, *Burkholderia* species deficient in QS showed massive die-off in the stationary phase of growth. Bacteria that metabolize amino acids as a carbon source release ammonia and increase the alkalinity of their environment, and this rise in pH killed the QS-deficient bacteria. The wild-type *Burkholderia* species maintained a lower environmental pH through the production and secretion of oxalate, a small acidic compound, and could thus survive the stationary phase. The production of oxalate—a so-called "public good" of benefit to all in the population—was activated through the QS system, indicating that the bacteria can sense their population density and, just before the stationary phase, cooperate to neutralize a toxic byproduct of their energy metabolism. — GR

Proc. Natl. Acad. Sci. U.S.A.

10.1073/pnas.1218092109 (2012).

NEUROSCIENCE

Misplaced Migration

Interneurons need to migrate from their birthplace to the cortical layers, where they will build their connections and support the effective function of cortical circuits. Disorders such as autism and schizophrenia that affect cortical circuits may also affect interneuron placement. A microdeletion at chromosome 22q11.2 has been correlated with risk of cortical circuit disorders. Meechan *et al.* used the *Large Deletion* 22q11.2DS (LgDel) mouse model to analyze what impact this microdeletion may have on cortical interneurons. In LgDel mice, a subset of the interneurons do not end up in the right place: During development, certain interneurons do not migrate as fast as normal and tend to lose their way. Analysis of transcription profiles identified a regulatory network affecting expression of the cytokine C-X-C chemokine receptor type 4 gene as a key defect in the malfunctioning interneurons. Interneurons carrying reduced amounts of cytokine receptor on their surface could be less responsive to migration signals and could thus easily end up in the wrong place. — PJH

Proc. Natl. Acad. Sci. U.S.A. **109**, 18601 (2012).



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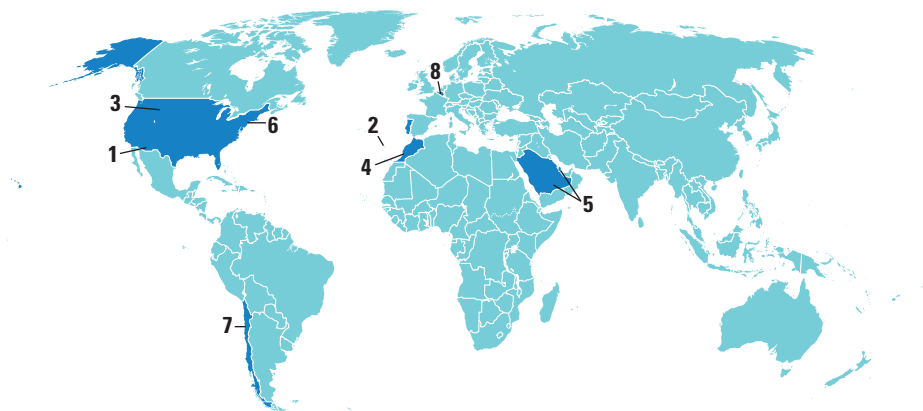
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AROUND THE WORLD



Tucson, Arizona 1

Biosphere 2 Launches Key Watershed Experiment

An iconic glass ziggurat in the desert, Biosphere 2 has shed its turbulent past and undergone a scientific rebirth over the past few years (*Science*, 8 July 2011, p. 146). This week, researchers began experiments with massive artificial watersheds on which they are betting Biosphere's future.

The 10-year experiment, called the Landscape Evolution Observatory, is designed to improve understanding of the



interactions of ecology, hydrology, and soils under climate change. It consists of three hillslopes, each 33 meters long and made of 650 tons of crushed volcanic rock. Researchers will measure how water and nutrients flow through soil using 1800 sensors embedded in each hillslope. Because the hillslopes are inside the Biosphere 2 domes, researchers can control temperature, precipitation, and light (but not the composition of the atmosphere, as Biosphere 2 is no longer airtight). "What makes it stand out is that it's big enough for interesting things to happen," says Gordon Grant, a hydrologist and geomorphologist with the U.S. Forest Service in Corvallis, Oregon, who is not involved in the project.

Madeira, Portugal 2

Dengue Outbreak Strikes European Outpost

An outbreak of dengue fever on the Portuguese island of Madeira, some 700 kilometers west of Morocco, has sickened more than 1300 people since 3 October. Dengue—which does not usually kill but causes crippling muscle and joint pains—occurs in many tropical and subtropical countries, but Europe has not seen sustained transmission since the 1920s, the European Centre for Disease Prevention and Control in Stockholm said last week. No deaths have been recorded, but 89 patients had to be hospitalized.

The dengue virus can be transmitted by several mosquito species; *Aedes aegypti*, the most effective vector, invaded Madeira in 2004 and has been spreading since, says Francis Schaffner, a parasitologist at the University of Zurich in Switzerland. Authorities initially tried to control the insects, "but it didn't work and they stopped," he says. *A. aegypti* does not occur in mainland Europe, but its cousin, *A. albopictus*—better known as the Asian tiger mosquito—is established in most Mediterranean countries and could act as a vector, albeit a weaker one, in the summer, Schaffner warns.

Yellowstone National Park 3

Hunters Kill Research Wolves

Hunters have killed an estimated 10 wolves from Yellowstone National Park this month, adversely affecting a park research program that has tracked the animals since their reintroduction in 1995. At least 88 wolves remain in Yellowstone, but the killings have "been a big hit to us scientifically," says project leader Douglas Smith. Particularly problematic, he says, is that seven of the



animals were wearing radio-tracking collars. Two "were the only collared members of their packs, so now we can't track those packs." Only one wolf wearing a specialized GPS collar is now left in the study. The killings, which were legal, occurred outside the park during the annual wolf hunting season that opened this fall in Montana, Idaho, and Wyoming. Some researchers fear hunters are targeting collared wolves (*Science*, 23 October 2009, p. 506), and have asked state officials to establish buffer zones around the park to protect the animals. Only Montana, however, has taken steps to protect wolves in one boundary region. <http://scim.ag/wolfkills>

Agadir, Morocco 4

Prospects Brighten for Shark Conservation

For the first time, the International Commission for the Conservation of Atlantic Tunas (ICCAT) agreed last week to consider explicitly including shark conservation in its mandate. "This is unprecedented," says Elizabeth Wilson of the Pew Environment Group, an environmental advocacy organization based in Washington, D.C.

ICCAT, which was established in 1966, manages some 30 migratory species, including swordfish, marlin, and other tunalike species. But the commission does not set catch limits for sharks. Most kinds of sharks are caught accidentally by vessels hunting for tuna and tunalike species, although a few



CREDITS (TOP TO BOTTOM): ISTOCKPHOTO; THE UNIVERSITY OF ARIZONA BIOSPHERE 2; DOUG PERRINNE, SEAPICS

Downloaded from www.sciencemag.org on November 29, 2012



The Secret of the Black Dahlia

Gardeners can choose from more than 20,000 varieties of dahlias, including whites, yellows, deep reds, and magentas. But especially alluring are the rarer black ones. Now, a team of researchers in Austria has turned the eye of science on what makes a dahlia black. The team collected 14 varieties of black dahlia—with names such as “Black Barbara,” “Arabian Night,” “Karma Choc” (left), and “Tisa” (right)—and five with tamer colors, then extensively analyzed their petals. They measured the activity of enzymes that make pigments, investigated gene expression, and measured the pigments themselves. Their conclusion: The black color comes from high levels of anthocyanins, the pigments that—at lower levels—also give orange and red dahlias their colors. The team reports in *BMC Plant Biology* that they think that most black dahlias raise their anthocyanin levels by blocking an enzyme in the pathway that makes flavones, another molecule that has the same precursor as anthocyanins. If scientists could figure out that trick, they might be able to engineer dahlias to make more black varieties.

species, such as shortfin makos, are targeted directly for their meat and large fins.

Although conservationists failed to win new protections for threatened sharks in the Atlantic Ocean at ICCAT’s annual meeting, which concluded last week, they hope to make significant progress over the next few years as changes to the treaty are negotiated.

Saudi Arabia and Qatar 5

Four More Cases of New Coronavirus Confirmed

Three new confirmed infections in Saudi Arabia—including a fatal one—and another in Qatar have prompted the World Health Organization (WHO) to urge countries to step up vigilance for a new coronavirus first reported in September. The four new cases bring the total to six, including two deaths, WHO said in a 23 November statement.

The new, as yet unnamed, virus is related to SARS; where it came from or how it spreads is a mystery. Two of the Saudi cases,

including the fatal one, were family members living in the same house. Lab tests are pending for another member of that household who also died and had similar symptoms; a fourth, nonfatal case in the same cluster tested negative. Countries should “consider testing” unexplained pneumonia cases for the new virus, WHO said, and thoroughly investigate such cases in doctors and nurses, who are at greater risk during disease outbreaks.

New York 6

Neurologist Implicated In Insider Trading Case

Federal authorities filed charges last week in what they said is the most lucrative insider trading case in history. According to the Securities and Exchange Commission (SEC), Sidney Gilman, a neurology professor at the University of Michigan, Ann Arbor, fed secret information about clinical trials of an Alzheimer’s drug to Mathew Martoma, a former portfolio manager at a division of the hedge fund SAC Capital Advisors, allowing

THEY SAID IT

“Government at all levels must recognize [the Sasquatch] as an indigenous people and immediately protect their human and Constitutional rights.”

—Melba S. Ketchum, director of DNA Diagnostics, in a press release on the firm’s unpublished claim to have sequenced the genome of the long-sought creature commonly called Bigfoot.

Martoma to make more than \$276 million in illicit profits and avoid losses with well-timed stock purchases and sales.

The drug, bapineuzumab, was a once-promising Alzheimer’s therapy originally developed by Elan and Wyeth. Based on information from Gilman, Martoma allegedly invested more than \$700 million in the companies’ stocks when the prospects for bapineuzumab looked good, and later unloaded more than \$960 million worth of stock in just over a week when the news turned bad.

SEC alleges that Gilman, a former chair of the Michigan neurology department who served as chair of the trial’s Safety and Monitoring Committee, received nearly \$108,000 for his consultations with Martoma from a New York-based firm that connects investors with technical experts. Gilman is cooperating with authorities in exchange for non-prosecution. <http://scim.ag/Martoma>

Atacama, Chile 7

New Director for ALMA

The Atacama Large Millimeter/submillimeter Array (ALMA) in Chile has appointed French astronomer Pierre Cox as its new director. Cox, an expert in millimeter and infrared astronomy, will take over ALMA’s stewardship in April 2013. More than half of the array’s 66 antennas are already in place on a high plateau in northern Chile, and the telescope has been taking observations for about a year. The complete array is expected to start operations by March 2013, right before Cox begins >>

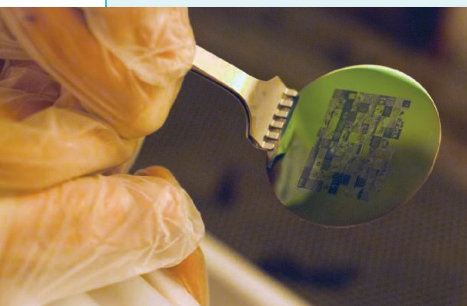


Random Sample

Art in Space

The longest-lasting contribution that Massachusetts Institute of Technology (MIT) graduate student Adam McCaughan will make in his promising career probably won't be a scientific paper or an invention. A message to aliens that he transcribed onto a metal disk last spring could outlast Earth itself.

American artist Trevor Paglen connected with MIT scientists in 2009, hoping they would help him launch tiny images, including a Texas dust storm and refugees experiencing the sea for the first time, on a 5-centimeter-wide disk into perpetual orbit. Paglen envisioned hyperevolved beings from our world or others as future recipients. Some of his inspiration for the grandiose project came from the plaque and audio record affixed to NASA probes Pioneer and Voyager, respectively. Both cosmic messages were etched into gold.



But MIT quantum nanostructures expert Karl Berggren noted that over the eons, grains in gold metal could shift and degrade the images. "We settled on [using] silicon with a layer of silicon oxide," says McCaughan, who transcribed the images using lithographic techniques used for making microchips. "No one really knows what happens in billions of years," Berggren told Paglen, "but this should work for a couple of hundred million." The 100 images on the disk require a microscope to be seen clearly and include depictions of human industry, oppression, technology, and climate change. MIT aerospace engineer Brian Wardle and astrophysicist Joel Weisberg at Carleton College in Northfield, Minnesota, designed a star chart as a protective cover.

The art piece was successfully launched on 20 November bolted to communications satellite EchoStar XVI. Both will orbit Earth at 38,000 kilometers.



>> AROUND THE WORLD

his tenure. Cox currently heads the Institute of Millimeter Radioastronomy in Grenoble, France. His knowledge of Spanish should serve him well in his new position.

Brussels 8

No Budget Set for E.U. Scientists

European researchers have to wait a bit longer to hear how much money they will have to spend in the coming years, but the outlook isn't bright. Last week, the leaders of all 27 E.U. member countries met in Brussels, yet failed to strike a deal for an overall budget agreement for 2014 through 2020. Still, the latest proposals for the "competitiveness for growth" budget, which includes research and education funding, call for up to 15% less than the €156 billion originally proposed by the European Commission, the European Union's executive branch. The downgrade stems primarily from E.U. countries lobbying for more agricultural and so-called cohesion funds. The state of

negotiations "does not bode well" for the research budget, says Helga Nowotny, president of the European Research Council. <http://scim.ag/noEUBudget>

FINDINGS

A Grand Old Canyon

Parts of Arizona's Grand Canyon may be millions of years older than previously thought, suggests a study of helium atoms in mineral samples from the western reaches of the canyon.

Helium, formed in minerals called apatites as a result of radioactive decay of uranium, diffuses through the mineral's crystal structure at deep underground temperatures above 70°C. But as the minerals rise toward Earth's surface—or as erosion carves a canyon toward the minerals—the rocks sur-



BY THE NUMBERS

20% Drop in new HIV infections worldwide since 2001, according to figures released by the Joint United Nations Programme on HIV/AIDS.

\$100 million Amount committed last week by AstraZeneca, Pfizer, and the Canadian province of Quebec to create the NEOMED Institute, a drug research facility in Montreal, Canada.

rounding them cool, trapping more of the helium within the apatite grains. Helium concentrations in apatite can, therefore, serve as a geological time clock.

The new study finds that much of the western portion of the Grand Canyon was carved to within a few hundred meters of its current depth by about 70 million years ago, scientists reported online this week in *Science*. That age is a far cry from the 5 to 6 million years previous researchers estimated by identifying when sediment from eastern portions of the canyon first appeared at the western end of the gorge. <http://scim.ag/oldcanyon>

Science LIVE

Join us on Thursday, 6 December, at 3 p.m. EST for a chat on **whether science can defeat influenza**. <http://scim.ag/science-live>



EUROPEAN SPACE AGENCY

Tough Financial Times Bring ESA Down to Earth

"It's been an interesting 2 days and 2 nights," said Jean-Jacques Dordain, director general of the European Space Agency (ESA), with impressive understatement. He was referring to an intense and often acrimonious council meeting in which ESA managers sought funding for their programs in many areas of space activity, while representatives of the 20 cash-strapped member governments tried to accommodate them, but with less money than ESA wanted.

The agency, at this ministerial council meeting in Naples, Italy, on 20 to 21 November, asked governments for roughly €12 billion over the 5 years from 2013 to 2017. By the end of the 48 hours, it came away with €10.1 billion (\$13.0 billion). As a result, Europe will be going to Mars, but not to the moon; it will collaborate with the United States to develop NASA's Orion Multi-Purpose Crew Vehicle; but it will have to wait for a decision on a next-generation Ariane 6 launcher. "It was a very difficult council ... with lots of stress and night discussions," Dordain said at the postmeeting press conference. "But by the standards of previous councils it was a big success, in spite of the economic situation."

Most ESA programs are optional for member states, so they can pledge as much or as little as they wish, and the process is rife with tactics and brinkmanship. U.K. science min-

ister David Willetts described it as "like trying to solve simultaneous equations and play poker at the same time."

One mission that fell victim to this process before the council even started was a proposed lunar lander. Championed by Germany, the small, 800-kilogram craft would have showcased automated landing technology by touching down near the moon's South Pole in 2018, where it would have probed the soil for minerals useful to future astronauts and monitored the environment for threats they might face. But by the week before the council, it was clear that not enough other members wanted to back the plan, so it was quietly shelved.

The Naples meeting started on a high note by giving the nod to collaboration with Russia on ExoMars, a program to send several craft to the Red Planet in 2016 and 2018. Originally a joint project with NASA, the U.S. agency pulled out of the deal earlier this year (*Science*, 24 February, p. 900). Russia has taken NASA's place.

Apart from the general budget that maintains ESA's headquarters and other facilities, the only program that all members are required to support is the highly regarded science program. The agency's plan for the next dozen years includes a mission to Jupiter's icy moons, a solar orbiter, and a dark energy mission. To carry these out, the science director-

Midlife crisis? Europe's aging workhorse Ariane 5 is facing new competition. Does it need a facelift, or a complete makeover?

ate wanted its budget to at least keep pace with inflation, but ministers sought to keep the program's budget for the next 5 years at the sum it received in 2012. Scientists in the United Kingdom are coping with flat budgets, Willetts says, and "it shouldn't be different for ESA."

Some argued to use the money provided by new members Poland and Romania to reduce all countries' contributions. ESA managers fought, successfully in the end, to add on the new members' contributions to the 2012 funding level and then keep it flat until 2017, giving the program €508 million per year, up from €480 million in 2012. With inflation eating away at that budget, "I'm afraid we will hear about a cut or delay of a mission," says Jean-Pierre Swings of the University of Liège in Belgium, chair of the European Science Foundation's European Space Sciences Committee.

Science was, however, a sideshow to the main dramas of the meeting, which involved launchers and the International Space Station (ISS). ESA's highly successful Ariane 5 rocket has dominated the commercial satellite launch market for many years, but its future is threatened by competitors, including the Falcon launchers of SpaceX and the Russian rockets. Modernization is needed, but while Germany favored an evolutionary approach, continuing the development of an upgraded version known as Ariane 5 ME (for "midlife evolution") with a new upper stage that can be relit to position multiple satellites, France wanted a more radical redesign—Ariane 6—which would reduce the cost per launch to keep it competitive.

The negotiations went on right through the night of 20 to 21 November with Belgium acting as go-between for France and Germany. The compromise essentially puts off the decision for 2 years: Ariane 5 ME development will continue while the ESA directorate puts together a more comprehensive proposal for Ariane 6 and looks for commonalities between the two that could reduce development costs. Ministers will meet again in early 2014 to decide whether to build one, the other, or both.

Earth observation, an optional program, similarly didn't receive all of the funding it had asked for and some proposed missions may have to be cancelled.

The other thorny issue was ESA's contribution to ISS, which currently comes partly in

the shape of its Automated Transfer Vehicles. Three of these crewless disposable freighters have already carried supplies to ISS, and another two are in the works. Some ESA members want to develop the technology further by transforming it into the “service module” for NASA’s planned Orion capsule. The detachable service module provides propulsion and control to Orion while it’s in space but would be jettisoned before the capsule descends to

Earth. Orion may carry four-person crews as far as the moon or an asteroid after 2020.

Developing and supplying the service module could form an in-kind contribution to NASA toward ESA’s ISS subscription for 2017 to 2020. But negotiations on how to fund the development fell short of the required amount until the United Kingdom stepped up as an unlikely savior. For decades, the United Kingdom has eschewed any involve-

ment in human spaceflight, but for this council Willetts came armed with more funding than usual (*Science*, 16 November, p. 868), of which he pledged €20 million for the service module. “British technology will now have an important role [in Orion],” Willetts says. “This is the first time ESA has contributed to a crew transport vehicle,” Dordain said. “For me that is a breakthrough.” An interesting 48 hours indeed.

—DANIEL CLERY

STRUCTURAL BIOLOGY

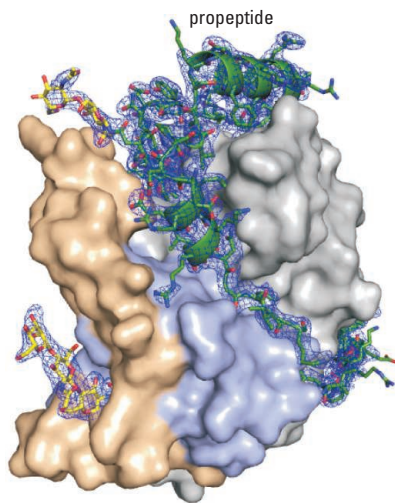
News Flash: X-ray Laser Produces First Protein Structure

For the first time, an ultraintense x-ray laser has revealed the previously unknown atomic-scale structure of a protein, researchers report online today in *Science* (scim.ag/Redecke). The advance ushers in a new type of protein crystallography. However, it’s too early to tell whether so-called x-ray free-electron lasers (XFELs) will supplant conventional x-ray sources known as synchrotrons, which have cranked out tens of thousands of protein structures, or merely serve niche applications, structural biologists say.

Researchers have determined the structure of an enzyme key to the survival of the single-celled parasite *Trypanosoma brucei*, which causes African sleeping sickness, a disease that kills 30,000 people each year. Scientists already knew the structure of the active form of the enzyme, known as a cysteine protease cathepsin. The new data reveal the enzyme’s “precursor” form, in which its active region is covered by a molecular safety cap called a propeptide, report Henry Chapman, a physicist at the German Electron Synchrotron laboratory (DESY) in Hamburg; Christian Betzel, a structural biologist at the University of Hamburg; and 47 colleagues. That information could help researchers find molecules that mimic the propeptide and tie up the enzyme, killing the parasite, Betzel says.

The way the structure was determined is as important as the result itself. To grow crystals of the enzyme, biologists over-expressed it in cells, where it formed elon-

gated crystals a fraction of a micrometer wide and several micrometers long. These crystals were too small to be studied with the relatively weak x-ray beams produced by circular particle accelerators known as synchrotrons. So the team dropped them through the beam of the world’s first XFEL, the Linac Coherent Light Source (LCLS) at SLAC National Accelerator Laboratory in Menlo Park, California, which is powered by a straight-shot linear accelerator.



Stopped. An x-ray laser revealed how this enzyme is bound up and deactivated.

Shining a billion times brighter than a synchrotron source, the LCLS delivers x-ray pulses lasting only a few millionths of a nanosecond. As in any x-ray crystallography setup, the x-rays scatter, or “diffract,” off the myriad planes of atoms in the complicated crystals, and the angles and intensities of the scattered x-rays reveal the crystal’s structure, including that of the protein. But unlike the pulses at synchrotrons, those at the LCLS are so intense that they can probe submicrometer-size crystals even as they blow them apart—an approach known as diffraction before destruction. Adding up 178,875 individual diffraction patterns, the researchers deciphered the structure.

Chapman and colleagues had previously shown that the LCLS, which powered up in 2009, could reproduce a known protein structure. Researchers at a laboratory called RIKEN SPring-8 Center in Hyogo, Japan, fired up their own XFEL earlier this year. Researchers at DESY plan to turn on the larger European XFEL facility in 2015.

Just how big the advance is remains unclear. The team did not determine the structure from the x-ray data alone but used as a starting point the structure of the enzyme’s active form. So the result marks a step toward such from-scratch, or de novo, structure determination, says Keith Moffat, a biophysicist at the University of Chicago in Illinois. “The real utility of the x-ray free-electron lasers is if they can determine structures that we simply can’t begin to determine with a synchrotron,” Moffat says.

However, the new work shows another advantage of an x-ray laser, says William Weis, a structural biologist at Stanford University in Palo Alto, California. The smallest crystals that can be studied at a synchrotron are damaged by the x-rays even as the data accumulate. Ironically, diffraction before destruction lets researchers glimpse a pristine crystal before it’s obliterated, Weis says. “If the XFEL is going to have broad impact, I think it’s going to be in doing damage-free data collection,” he says.

Then there’s the matter of beam time. A synchrotron serves dozens of users simultaneously. The LCLS serves one user at a time, and even the European XFEL will likely serve at most 10 at once. So even researchers on the same team disagree on whether the XFEL will vie with the synchrotron for business. “I see it as an add-on that allows you to look at specific cases such as membrane proteins that won’t crystallize very well,” Betzel says. But Chapman says the lasers may be able to do things that synchrotrons cannot, such as study rapid changes in molecules. “Why wouldn’t they [draw away synchrotron users] if everything is so much better?” he says.

For researchers working on XFELs, the dream is to determine protein structures by zapping individual molecules. Weis and Moffat say it’s not yet clear that can be achieved.

—ADRIAN CHO

GULF OIL SPILL

BP Criminal Case Generates Record Payout for Science and Restoration

Ecologists working along the Gulf of Mexico have plenty of ideas for restoring the region's deteriorating wetlands and fisheries. But for decades they've been frustrated by a lack of money to implement them.

Their spirits got a dramatic lift on 15 November, however, when petroleum giant BP announced that it had agreed to pay an unprecedented \$4 billion to settle criminal charges related to the 2010 *Deepwater Horizon* oil spill. Some \$2.5 billion is dedicated to jump-starting ecological restoration projects in the five gulf states. Another \$350 million will go to the U.S. National Academy of Sciences (NAS) to create an unusual regional research effort that will run for 3 decades. The settlement is the latest in a string of spill-related payouts that could ultimately pump more than \$20 billion into gulf restoration and science.

The BP money "is probably the best chance we will ever get to restore this vibrant ecosystem," says Jeff Trandahl, executive director of the National Fish and Wildlife Foundation (NFWF), a Washington, D.C.-based philanthropy that discovered only from press reports that it would be managing \$2.394 billion of the restoration money. (Another \$100 million will go to the North American Wetlands Conservation Fund.) "We were a little shocked," he says.

NFWF, created by Congress in 1984 to promote public-private cooperation on conservation, spent \$130 million last year on about 570 projects nationwide. NAS, which Congress created in 1862 to advise the government on technical issues, has annual revenues of \$325 million from public and private sources. Officials at both organizations say attorneys from the U.S. Department of Justice approached them earlier this year to outline the BP settlement.

A federal judge is expected to approve the payments, which will be made over 5 years, within a few weeks. Meanwhile, both groups are figuring out how best to use the windfall. The academy, which typically conducts studies that synthesize existing information, will be thrust into the more unusual role of funding original research. "That's not unprecedented," notes NAS spokesman William Skane. The U.S. National Academies' Transportation Research Board, for instance, already helps run a \$60-million-a-year grants program.

It isn't yet clear how much money the gulf program will set aside for grants, or how they will be awarded, Skane says. But the settlement specifies that studies should focus on understanding and reducing the human health and environmental risks posed by oil production. It also calls for NAS to fund education and training programs, and efforts to improve environmental monitoring.

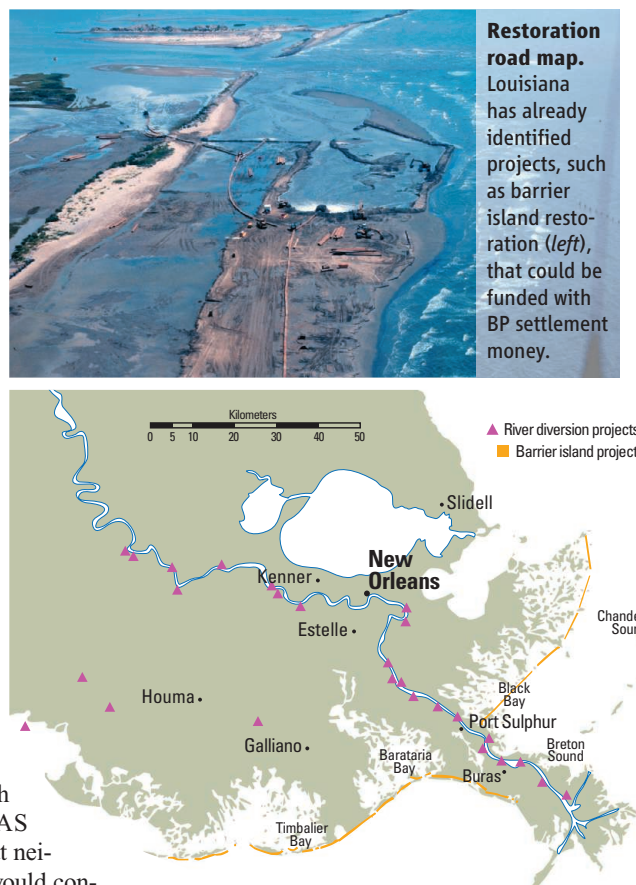
"We can use these funds to help fill some really significant gaps in the science being done in the gulf," says NAS Vice President Barbara Schaal, a plant geneticist at Washington University in St. Louis. One critical need, she says, is to develop large-scale, long-term monitoring systems for the gulf ecosystem. Although the academy won't have enough money to single-handedly build such systems, she says it can help identify how "to do monitoring in an appropriate, modern way." That work could begin soon: The academy will get its first \$5 million payment within 90 days of settlement approval. It must spend the entire \$350 million—plus any interest it earns—within 30 years.

During negotiations with the Justice Department, NAS officials wanted to be sure that neither the government nor BP would control how the money is spent (they won't), and that there is "scientific merit" to the issues they are being asked to address (there is, they decided). And although \$350 million "sounds like a lot," Schaal says, the fact that the money will be spent over 30 years means it won't dwarf the academy's other programs.

At NFWF, which typically receives up to \$10 million a year from fines and penalties generated by federal environmental crime cases, officials are bracing to run the group's largest effort ever. Hard-hit Louisiana gets 50% of the money, specifically to "create or restore barrier islands off the coast" and reengineer the lower Mississippi River to restore water and sediment to degraded wet-

lands. Three states—Alabama, Florida, and Mississippi—will each get 14% for projects that "remedy harm" from the oil spill and "reduce the risk of future harm." Another 8% goes to Texas.

Louisiana officials say they already have plenty of shovel-ready projects. In May, state lawmakers approved a 50-year restoration road map that identifies more than 100 high-priority projects that could cost some \$50 billion. Planners can't say which will go first, but NFWF Vice President of Conservation Projects Thomas Kelsch says decades of research went into the road map, mean-



Restoration road map. Louisiana has already identified projects, such as barrier island restoration (left), that could be funded with BP settlement money.

ing "we'll be making investments based on the best available science." Planning is less advanced in the other gulf states, however.

NFWF and NAS are also figuring out how to fit in with numerous other spill-related programs. BP has promised \$500 million over 10 years to the Gulf of Mexico Research Initiative, which is funding science consortia, and \$1 billion to restore habitat directly damaged by the spill. And BP and other companies still face lawsuits that are expected to funnel another \$5 billion to \$20 billion to restoration and research. "It would be really sad," Schaal says, to have several organizations "doing the same thing and then not have a major area covered." —DAVID MALAKOFF



One more chip. West Antarctica is losing ice at an accelerating rate as glaciers rush to the sea.

GLACIOLOGY

Experts Agree Global Warming Is Melting the World Rapidly

Glaciologists are still far from divining the fate of Earth's ice in a warming world, but they have finally agreed on what the past century's warming has done to the great ice sheets, and it isn't pretty. Researchers had been sizing up the millions of cubic kilometers of ice stored in Greenland and Antarctica using four different techniques applied to different regions at different times, but they just weren't getting it together. So 47 experts put their heads together over all the data to arrive at a community consensus.

The globe's icy bottom line: a current annual loss of 344 billion tons of glacial ice, accounting for 20% of current sea level rise. Greenland's share—about 263 billion tons—is roughly what most researchers expected, but Antarctica's represents the first agreement on a rate that had ranged from a far larger loss to an actual gain. The new analysis, published on page 1183 of this issue, also makes it clear that losses from Greenland and West Antarctica have been accelerating, showing that some ice sheets are disconcertingly sensitive to warming.

"They got a number of heavy hitters in the field to sit down and agree that the numbers agree," says glaciologist Richard Alley of Pennsylvania State University, University Park, who was not involved in the work. "To me, that says they know what they're doing. And that's a very important step for forecasting ice's behavior in the future."

It hadn't always been clear that researchers in the sometimes fractious field of gauging ice sheets knew what they were doing. In trying to measure ice sheet changes of 1 part in 100,000 per year, small errors loomed large. "There were so many numbers published out there, how could one not get confused?" says glaciologist Robert

Bindschadler of NASA's Goddard Space Flight Center in Greenbelt, Maryland. In the past 15 years, at least 29 studies have estimated how quickly the ice sheets had been losing or gaining mass. The numbers were all over the map, allowing an overall change in ice sheet mass ranging from a loss of a whopping 676 billion tons per year to a gain of 69 billion tons (*Science*, 22 July 2011, p. 401).

In 2011, scientists working on the upcoming climate assessment by the Intergovernmental Panel on Climate Change decided someone had to try something new. The result was the Ice Sheet Mass Balance Intercomparison Exercise (IMBIE) supported primarily by NASA and the European Space Agency, the agencies that fly the satellites that had returned all that confusing data.

IMBIE's 47 participants from 26 institutions—headed by glaciologists Andrew Shepherd of the University of Leeds in the United Kingdom and Erik Ivins of NASA's Jet Propulsion Laboratory in Pasadena, California—tried to make sense of published changes in the ice sheets that were based on four different techniques. Each technique gauges the changing amount of ice as snowfall adds ice to an ice sheet and melting and glacier flow to the sea removes it.

In two of the techniques, a satellite repeatedly bounces a radar or laser signal off an ice sheet to measure its changing height and thus its changing volume. The Gravity Recovery and Climate Experiment (GRACE) mission estimates ice sheet mass by measuring the changing pull of gravity on its two satellites as one and then the other passes over. And the one nonsatellite technique depends on regional climate models to estimate input from snowfall and losses from melting. This input-output method also requires satellite

radar measurements of the speed of flowing glaciers.

In the end, reconciling the diverse ice-loss estimates proved to be more straightforward than had been feared. It turned out that gains and losses of ice can vary greatly from season to season and from place to place. So surveys made over different, albeit overlapping,

time periods and regions yielded rather different loss rates. Once the data were adjusted to uniform regions and periods and a few other modifications were made, "there's no reason to believe the data sets are saying different things at all," Shepherd says. "They're showing the same thing."

By the new reckoning, the Greenland ice sheet lost 263 ± 30 billion tons of ice per year from 2005 to 2010. Overall, Antarctica lost about 81 billion tons per year in the same period; the huge East Antarctic portion of the ice sheet registered a small gain, more than offset by losses in West Antarctica and the adjacent Antarctic Peninsula. Since 1992, the two ice sheets lost enough ice to raise sea level by about 0.6 millimeters per year on average, out of the observed 3 millimeters per year. (Most of the rest of the sea level rise came from melting mountain glaciers and from the expansion of seawater due to warming.)

The IMBIE results "represent a maturation of the community," Bindschadler says. "Before, groups were somewhat antagonistic toward each other; this represents a willingness to come together. It reconfirms that these changes in the ice sheets are real. The giants of the West Antarctic and Greenland ice sheets are waking up to changing climate at an increasing rate."

Glaciologists are especially concerned about the acceleration of losses. The acceleration in the north shows that "Greenland is pretty sensitive to [air] temperature," Alley says. "If we make it too hot, Greenland is in real danger of melting away." In West Antarctica, the accelerating loss comes from the accelerating rush of glaciers to the sea, probably brought on by warmer seawater melting the underside of the glaciers' floating ice shelves. "That shows the real sensitivity of these [glacier] flows to ocean temperature," Alley adds. The next chore for glaciologists is to incorporate this new understanding into models that can predict future ice sheet behavior in a warming world. None can today.

—RICHARD A. KERR

OIL RESOURCES

An Oil Gusher in the Offing, but Will It Be Enough?

Some stunning headlines followed the International Energy Agency's (IEA's) release earlier this month of its *World Energy Outlook 2012*. "U.S. Oil Output to Overtake Saudi Arabia's by 2020," blared Bloomberg, for example. That may be true, but the more significant aspect of the *Outlook's* projections was the prospect for world oil. Under the right conditions, the report says, the world could produce increasing amounts of oil right through 2035 and meet the world's growing demand for energy as oil.

The catch is "under the right conditions." Everyone agrees that the oil is out there. The trick will be wresting it from the ground under difficult circumstances as fast as the world needs it. The United States would have to triple its production of so-called tight oil, requiring tens of thousands of new hydrofractured wells. Fragile Iraq would have to triple its current production. And the world would have to figure out how to run motor vehicles on a sort of petroleum gas currently of little or no use in transportation. As IEA's chief economist, Fatih Birol, says of the Iraq situation, "there are many challenges."

The challenges are there because, according to IEA, the world will never again produce crude oil—the familiar black goo that pours from a well with little or no encouragement—as fast as it did in 2005 at the peak of crude production. If drillers frantically drain currently producing fields, develop known fields, and find new ones, they can only hope to keep crude oil production roughly level until 2035. No increase in crude is coming.

In the *Outlook's* featured scenario, increasing population and rising standards of living push the demand for oil from 2011's 87.4 million barrels per day to 99.7 mb/d in 2035. In this scenario, to help meet that increased demand, oil-producing countries would have to double their production of so-called unconventional oil. That's oil locked up in rock or sand so tightly it won't come out of a well on its own, like U.S. tight oil trapped in nearly impermeable rock or Canada's tarry oil stuck to sands. These unconventional oils

are abundant, but tight oil requires hydraulic fracturing of the rock, and oil sands need to be steamed underground or bodily dug up and processed.

Only high oil prices make such efforts worthwhile, oil analyst Richard Nehring of Nehring Associates in Colorado Springs, Colorado, notes. With the high prices of recent years, U.S. tight oil production has soared from next to nothing to almost 1 mb/d, mainly from North Dakota. In the *Outlook* scenario, U.S. tight oil production continues its steep ascent toward 3.2 mb/d in 2020. "That's in the range of feasibility," Nehring says. If that happened, it would help put the United States ahead of Saudi Arabia and prop up world oil production.

But it will take more than continued high oil prices for unconventional to help save the day. As tight oil production rises further, "the drilling rates get interesting," says oil analyst Richard Miller of Addlestone, U.K. Any

end up in gasoline, plastics, and your backyard gas barbecue.

Lately, though, many energy organizations have been lumping NGLs in with crude oil and calling it "liquids" or, as IEA does, just plain oil. IEA does adjust its scenario's 50% increase in NGL production by 2035 to account for the 40% lower energy content of NGLs compared with crude oil. But that does not entirely reflect the way NGLs are used today. Fifty-four percent goes into petrochemicals, according to an analysis by Anne Keller of Wood Mackenzie in Houston, Texas. Only 17% ends up in gasoline for transportation, and only 19% is burned as fuel. If NGLs are to meet a growing demand for transportation fuel, especially diesel, their processing will have to be rejiggered somehow.

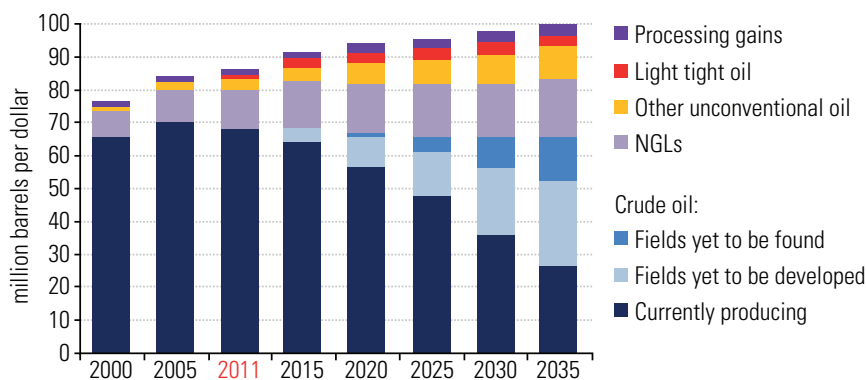
Finally, the IEA scenario calls for Iraq's oil production to triple. "Geologically, it's clearly possible," Nehring says, "but it's everything else that's problematic," as IEA points out in

some detail. To increase its current production of 2.6 mb/d to 8.3 mb/d in 2035—more than double its previous production high—Iraq would have to consolidate its shaky political stability, invest billions to supply water to stimulate production, and "remove impediments to investment," among other challenges. Things are already looking tough in the investment arena. Earlier this month, Exxon Mobil told Iraq

that it wants to withdraw from a \$50 billion oil project there, reportedly because the country's increasingly restrictive fiscal terms for the deal meant Exxon Mobil would not be making any money.

Indeed, money may be the most uncertain factor in the IEA scenario. It has the price of a barrel of oil rising to \$125 in real terms by 2035. Such an increase would fund the maintenance of crude oil production, drive up production of unconventional oil, and encourage transportation's shift toward NGLs. The catch: The Organization of the Petroleum Exporting Countries would have to restrain its production as non-OPEC production surges to allow prices to rise. The scenario is silent on the chances of that.

—RICHARD A. KERR



It's a plan, anyway. IEA's scenario has world crude oil production (blues on bottom) nearly holding steady, a possibly iffy expectation. Increasing demand would be met by expensive unconventional oils (red and yellow), but NGLs (lavender) would be needed, too.

new oil well's output peaks and then goes into decline, but tight-oil well production peaks quickly and drops precipitously, 40% to 80% during the first year of production. That means lots of new, expensive wells need to be drilled into large volumes of oil-rich rock. But estimates of the amount of accessible tight oil "are poorly known as of now," Birol notes.

Tight oil alone won't meet rising demand, of course; more natural gas liquids (NGLs) will be needed. Actually liquid only when pressurized or chilled, NGLs are the hydrocarbons that fall between the methane of natural gas, the lightest hydrocarbon, and the larger molecules heavy enough to be included in crude oil. NGLs are mostly a byproduct of natural gas production; they



Raising Up a Fallen Ivory Tower

Myanmar's universities, benighted after decades of isolation and neglect, are striving to make up lost ground and recover lost student bodies

YANGON, MYANMAR—When Ronald Daniels became one of the first Americans in many years to set foot on the campus of Yangon University in January, it should have been a moment to savor. Instead, says the president of Johns Hopkins University in Baltimore, Maryland, the experience was “heart-wrenching.” Before Burma’s military staged a coup d’état in 1962, Rangoon University, as it was then known, “was one of the storied institutions of higher education,” he says. But to the junta, the university was a recurring headache. After crushing protest after protest there, in 1996 the generals banished undergraduates to campuses in the countryside, where they could be kept under surveillance more easily. Higher education spiraled into an abyss.

Today, Yangon University and its charming colonial-era buildings are “basically a ghost town,” Daniels says. “The university has become a powerful metaphor for what happened to the intellectual capacity of the country.”

But Myanmar’s political transformation is giving academics hope that their long, dark night is nearing an end. President Thein Sein, a former general who took office in March 2011, has reintroduced press freedoms, canceled a massive Chinese-led dam project, and released opposition leader and Nobel laureate Daw Aung San Suu Kyi from house arrest.

Now, higher education is about to take center stage. Next week, the country’s top engineering institutions—Yangon Technological University (YTU) and Mandalay Technological University—will admit their first undergrads in more than a decade. “This is a turning point,” says civil engineer Nyi Hla Nge, a former YTU rector. Other universities are slashing enrollment to boost faculty-student ratios and renovating threadbare labs. “We accept that we’re at least 20 years behind our Southeast Asian neighbors,” says Tint Swe Latt, rector of University of Medicine 2 here.



Like-minded. Aung San Suu Kyi and Johns Hopkins President Ronald Daniels want to see Yangon University restored to former glory.

Sprucing up. Yangon Tech prepares for its first crop of undergrads in a decade.

Myanmar’s downtrodden academics got a boost last week from Barack Obama, whose brief stay here was the first by a U.S. president and a milestone on the Southeast Asian nation’s rapid transformation from pariah to budding democracy. In a speech at Yangon University on 19 November, Obama urged the nation to revive what had once been one of Asia’s premier centers of learning. Yangon University, he said, “must reclaim its greatness, because the future of this country will be determined by the education of its youth.”

Under the junta’s brutal reign, health care eroded, ethnic strife exploded, and civil liberties were curtailed. All of this left deep scars in the Burmese psyche. “There’s been no incentive to study since 1962,” says petroleum geologist Soe Myint, president of the Myanmar Geosciences Society. After decades of repression, he says, “few people dare speak freely. That mindset is not something you can change overnight.” Adds Sayama,* a senior professor at the University of Medicine 1 here, “Our people have been closed up so long, they don’t know what it’s like outside.”

Restoring Myanmar’s higher education system “is only going to work if universities are seen as an indispensable asset to the country,” Daniels says. “Ultimately the real test is: Are undergraduates going to be welcomed back to Yangon University?” Aung San Suu Kyi, now a legislator in Myanmar’s increasingly assertive parliament, has taken up that cause. “[O]ur education system has gone in the utterly wrong direction,” she declared in parliament earlier this month, according to *The Myanmar Times*. Overriding the education ministry’s objections, legislators passed a proposal from her to form a panel to oversee Yangon University’s revitalization, including the return of undergraduates. That momentous step may encounter resistance. With nearly 5000 graduate students at Yangon University, “we have no room for undergraduates,” insists physicist and vice rector Pho Kaung.

A moment of truth for Burmese society has arrived. “Some of my colleagues think we are doing OK. But we’re not OK, we’re far behind,” Sayama says. “We need to catch up in a hurry.”

Into the abyss

Sayama was 10 years old when a thunderous explosion in the early morning on 7 July 1962 caved in the roof of her apartment. Security

*Pseudonym

forces had just blown up Rangoon University's student union building. "It was terrifying," she recalls. Her parents lived on campus; students had been vociferously denouncing the coup of the previous March. Tanks rolled onto the Rangoon University campus that day, and dozens of students died. It was the beginning of the slow strangulation of Burma's academic life.

As Burmese society turned inward, Sayama was one of the lucky ones sent abroad by the government at that time. She won a scholarship to study at Royal Postgraduate Medical School in London. After earning a Ph.D. in 1990, she could have remained in London for postdoctoral research. "I was happy there," she says. But a few decades earlier, her father, a Georgetown University graduate, had returned to Burma. Sayama couldn't abandon her parents or her country. "If I didn't come back," she says, "I'd break their hearts."

While she was overseas, the atmosphere in Burma had grown even more toxic. In 1988, the military viciously cracked down on pro-democracy protesters, killing thousands, and declared martial law. Back at the University of Medicine 1 in 1990, Sayama was frustrated. The junta disdained foreign ideas; speaking English was frowned upon. "We were so out of date," she says. But when she warned that the rest of the world was leaving Myanmar behind and suggested ways the university could modernize, colleagues complained that they didn't need "some highfalutin person telling them what to do."

Like their compatriots at Yangon University, medical students denounced the junta, and some died in clashes with soldiers. The country's four medical universities got to keep their undergrads, with the proviso that they could not live on campus. (They hope to reopen dorms "in the next few years," Tint Swe Latt says.) The regime understood that disrupting the medical schools "could decrease teaching standards and cost a lot of lives," says Than Cho, rector of University of Medicine 1.

Although the medical universities enjoyed a measure of protection, they ended up in the same plight as Yangon University: impoverished and desperate. On campuses across Myanmar, Western-led sanctions and meager budgets have precluded properly outfitting labs. When universities managed to obtain an instrument, Than Cho says, "if it arrived damaged, there was nothing we could do," as sanctions made it virtually impossible to obtain spare parts or get technical assistance. As part of a national 30-year education plan adopted in 2001, Yangon University acquired \$2 million worth of instruments from Japan, includ-

ing an x-ray diffraction spectrometer and a scanning electron microscope; now, five of the 15 machines are broken and several others are deteriorating fast. Nationwide, universities routinely download textbooks from the Internet and distribute bootleg copies. "The students are very poor, so we cannot comply with copyright law," Nyi Hla Nge says. "We can't stand on our feet now. We need help."

The isolated campuses built in the boondocks for undergrads are also hurting. The big-



Painful past. Yangon University's Pho Kaung (left) does not know when undergrads will return to his campus. Restoring Myanmar's higher education system "is a task for Superman," Soe Myint says.

gest is Dagon University, 20 kilometers north of the city center. Some 24,000 students make the trek to the campus for classes. Another 40,000 take courses by computer and show up for exams. (The junta preferred distance learning, which constrains restive youth from mingling.) When *Science* visited on a Thursday morning earlier this month, Dagon's barren teaching labs and empty classrooms with barred windows were not nearly as inviting as its outdoor canteens thronged with students.

Poorly equipped as its facilities are, Dagon does have an academic pulse. The university is the only one in Myanmar where students

Online

sciencemag.org



Podcast interview
with author Richard
Stone (http://scim.ag/pod_6111).

For example, zoology department chair Khin Maung Swe has co-authored peer-reviewed articles on the world's smallest mammal, the Kitti's hog-nosed bat. Also known as the bumblebee bat, the bantam creature—it weighs less than 2 grams—has been recorded in three caves in southeastern Myanmar since 2001.

For scientists, Myanmar's remote forests are among the last remaining terra novae.

In the January 2011 issue of the *American Journal of Primatology*, Burmese and foreign researchers unveiled a new primate, the Burmese snub-nosed monkey (*Rhinopithecus strykeri*), discovered in northeastern Myanmar. Cash-strapped academics here are eager to lead expeditions—if foreign colleagues foot the bill. "We have no money for fieldwork," says Khin Maung Swe. Seizing the opportunity, the Chinese Academy of Sciences is funding a 2-week bilateral foray



across Myanmar that is now in progress. They are collecting rocks for tectonic, paleoclimate, and sedimentary studies.

Seizing the initiative

In the early 1950s, Burma, emerging from decades of British rule, sent dozens of top students to the Massachusetts Institute of Technology (MIT) for graduate studies. Returning home, the elite scholars became assistant professors at Rangoon Technological University, later known as YTU. "They were pioneers of an American system of education," Nyi Hla Nge recalls. "The intention was to create an MIT of the East."

YTU was poised for a revival in the late 1990s, when Nyi Hla Nge was rector. It launched Ph.D. programs in 1997 as the first step toward becoming a comprehensive S&T university. That plan foundered in 2000 when the junta appointed an army officer as science minister. YTU was stripped of undergrads in 2001 and "almost closed," Nyi Hla Nge says. The new minister promptly went on a building spree, opening several universities and technical colleges that mirrored the education ministry's undergraduate universities. As enrollments soared in the new schools, teaching standards plummeted, Nyi Hla Nge says: "Professors taught long hours without rest." In the last decade, Myanmar's 42 technologi-

cal universities have churned out tens of thousands of engineers and technicians, most of whom are poorly qualified, according to the companies who hired them, Nyi Hla Nge says.

When political reforms started to take root here last year, Nyi Hla Nge and industry minister Aye Myint, an electrical engineer by training and YTU alumnus, swung into action. “We tried many different paths” to persuade authorities to resurrect YTU, he says. Their persistence paid off late last summer, when Thein Sein gave YTU and Mandalay Tech the green light to bring back undergrads. Each will take in 250.

YTU faculty members have been busy ginning up a curriculum that accounts for eroded education standards. Freshmen at YTU and Mandalay will take a required “foundation” course to remedy poor secondary schooling, then another 5 years of coursework to complete a 6-year bachelor degree in engineering: “maybe the longest in the world,” says Nyi Hla Nge, who has been busily writing lecture notes in five subjects. The goal, he says, is that by 2020, YTU’s graduates will be as competent as those of Nanyang Technology University in Singapore and other top schools in the region. A wealthy YTU alumnus has donated materials for a physics teaching laboratory, and when *Science* visited, YTU’s main building was getting a facelift to welcome its first crop of undergrads since 2001. “We’re very proud,” Nyi Hla Nge says. “It’s a golden chance.”

Prospects are also improving at the University of Medicine 1. It’s about to build a molecular biology lab thanks to a \$300,000 grant from the China Medical Board, a foundation based in Cambridge, Massachusetts, and health ministry funds. For the first time, Sayama says, “we can buy our own primers, our own gel electrophoresis machines.” The four medical universities together will boost the professor-student ratio next year by slashing freshman enrollment in half, to 1200 students. “This will make teaching more effective,” Than Cho says.

A job for Superman?

Not long ago, a normal academic life in Myanmar was unimaginable. Until this year, “we would have foreign visitors arrive at our gate, but we could not get permission from the government to let them in,” says Myo Win, rector of the University of Dental Medicine here. “For the past 50 years,” says Tint Swe Latt, “we had a closed-door policy.”

Universities now have the right to host whomever they please, as long as they notify their respective ministries. (Thirteen ministries oversee Myanmar’s 156 universities.) But any significant reforms still require



Thinking big. Nyi Hla Nge dreams of establishing an “MIT of the East” in Yangon.

approval from the capital, Naypyitaw. Parliament is drafting a law intended to grant academia more independence and open the door to private universities. Negotiations have been tricky. “Autonomy for universities is a dangerous concept for the government,” says David Maynard, deputy director of the British Council office here. Wrangling over the law’s scope suggests it will not materialize until late 2013 at the earliest, observers say.

Proceeding in parallel is a top-to-bottom review of the entire education system. Launched last month by the education ministry, the 2-year-long Myanmar Comprehensive Education Sector Review “is our own form of educational peace-building,” says Maurice Robson, CESR’s international coordinator. Already, he says, “people have a well-developed sense of what is not working.” One systemic flaw is that young people here receive 11 years of primary and secondary schooling, 1 year less than in many other countries. “That’s a significant problem,” Robson says. Another issue is the dropout rate. Only half of children in Myanmar enroll in middle school, and just 11% go on to university. Higher education, Robson says, “will be elitist for quite a while.”

Remedying the education system’s many woes won’t be easy. “After such a long period of atrophy, where do you start?” Maynard asks. Soe Myint puts it this way: “This is a task for Superman.” The government doubled the education ministry’s budget in 2012 and has pledged to double it again, to 8% of GDP—as much as \$1.5 billion—next year. One investment that would quickly pay dividends is a robust university cyber-network. Better hardware and more bandwidth would enable Burmese researchers “to document and publish more local data and publish more

co-authored papers with other scientists,” says Steven Huter, director of the Network Startup Resource Center at the University of Oregon in Eugene. Huter met government officials and university scientists in Myanmar last May to discuss practical steps for building a national research and education network.

Daniels returned from his trip to Myanmar with a sense of what it will take for Burmese academia to recover. “It’s a long road back. The physical regeneration of the campuses will not be trivial.” Johns Hopkins is reviving a link with Yangon that dates to 1954, when it established the Rangoon-Hopkins Center for Southeast Asian Studies at Rangoon University. Johns Hopkins medical professors are making frequent trips to Myanmar “to engage them in health problems” such as HIV/AIDS, Daniels says. The university has also established graduate fellowships for Burmese students. Success of the overall enterprise of rehabilitating Myanmar’s universities may depend on the deepening of political reforms. “It’s up to the government to commit to the idea that a university is a core institution of Burmese society,” Daniels says.

Thein Sein has signaled that he is ready to embrace that idea. He told United Nations Educational, Scientific and Cultural Organization Director-General Irina Bokova last



August that Myanmar “badly needs support in higher education reform and strengthening universities,” says Sardar Umar Alam, project manager of UNESCO’s Myanmar Education Recovery Programme in Yangon. He suggests that Myanmar start with pilot models and experiments: Reform a few departments in three or four universities.

That’s the approach the science ministry is taking with YTU and Mandalay Tech. The glasnost spreading through Myanmar has even rekindled Nyi Hla Nge’s desire to establish, someday, an MIT of the East. “Our dream is to revive that,” he says. “I would like to dispatch our young teachers to the United States again.”

—RICHARD STONE

PHYSIOLOGICAL ECOLOGY

Salt and the Sea Serpent

Despite millions of years living in the ocean, sea snakes still have to watch their salt intake

In 2009, Harvey Lillywhite wanted to know if true sea snakes got thirsty. To catch some, his research team angled a small motorboat toward a distinctive band of water caused by two converging currents in the Papagayo Gulf in Costa Rica. Sneaking up on his quarry, a yellow-bellied sea snake floating on the surface, he gently slipped the venomous animal into a net and then into a bucket. After collecting a dozen or more snakes this way, the physiological ecologist at the University of Florida in Gainesville went back to a temporary lab to test each one. He laid the first one on a towel. After its skin had become dry to the touch, Lillywhite weighed the snake and dropped it into fresh water.

Soon the snake opened its mouth and began gulping down water, increasing its body weight by 13% by the next morning, Lillywhite recalls. He has since observed the same behavior in scores of sea snakes collected over the past few years. But, contrary to decades of academic thinking that sea snakes can thrive on seawater, none would drink salt water that Lillywhite provided—no matter how dehydrated they got. When it comes to sea snakes, the textbooks are wrong, Lillywhite asserts.

Although sea snakes seem exquisitely adapted to the marine environment, with a flattened body, paddle-shaped tail, and glands that secrete salt, salt still dictates where, how, and perhaps even if they live, Lillywhite and his colleagues have found. Their recent study of the global distribution of these reptiles, for example, has revealed that salinity has limited the abundance and distribution of these species. “People assumed that [sea snakes] have salt glands and drink seawater, and that’s all there is to it,” says William Dunson, a retired physiologist in Englewood, Florida, who studied the reptiles. “But that’s not the way it is.”

Like marine birds and marine mammals, snakes in the sea evolved from terrestrial ancestors, and the transition required adjusting to a life of avoiding the intake of too much salt and the loss of too much water. Textbooks have long said that salt glands solved this problem for marine birds and reptiles by removing excess salt from ingested seawater.

Yet Dunson notes that his surveys of sea snakes in the 1970s revealed that their salt glands were often very tiny compared with

those in other marine animals. That and other observations led him to conclude that the snakes “probably didn’t drink [seawater] except when salinity was very low.”

Lillywhite himself began to seriously question the dogma in the 1990s after he discovered that a marine snake unrelated to sea snakes required fresh water for survival. He then took a look at sea kraits, which differ from true sea snakes in that they move to land to digest their food and lay their eggs. (True sea snakes never leave seawater and give birth to live young.) Even when dehydrated, the sea kraits refused to drink seawater but lapped up fresh water, Lillywhite and his colleagues reported in 2008. On Orchid Island in Taiwan, sea kraits were also far more abundant close to freshwater springs or rivers entering the oceans and in years when there was high rainfall.

Others were also becoming suspicious that sea kraits couldn’t survive on seawater. Xavier Bonnet and François Brischoux at the CNRS Chizé Centre for Biological Studies in Villiers en Bois, France, have observed that in dry periods, sea kraits cease to hunt in water and instead hide on land, waiting to emerge en masse to drink puddled rainwater on rocks when the dry spell breaks. “They just have not been able to adapt to the salinity, and they still depend on fresh water,” says John Murphy of The Field Museum in Chicago, Illinois, who studies snakes.

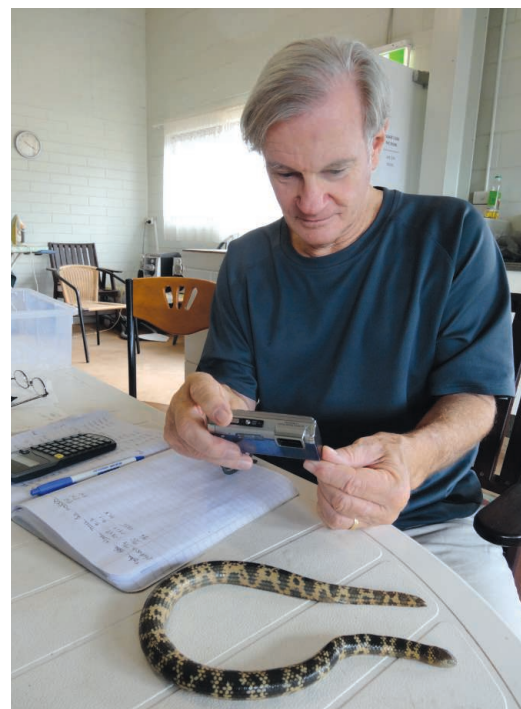
The 60 or so species of true sea snakes live permanently in the ocean, and although there had been some suggestion that they can drink lenses of fresh water that pool on top of the denser salt water, it was unclear whether they needed this supply to survive. To resolve this issue, Lillywhite has been taking periodic trips to Costa Rica, with visits that span the dry and wet seasons. Yellow-bellied sea snakes, like sea kraits, dehydrate in seawater. When thirsty enough, they drink fresh water, never salt water, Lillywhite’s team reported in the August issue of *Integrative and Comparative Biology*.

The fieldwork also indicated that these sea snakes don’t migrate to rivers or estuaries during the dry season. Instead, they likely

take their chances that a good rain will leave fresh water on the sea surface. Yet, Lillywhite notes, “even in the wet season, the storms can be sporadic and spotty.”

Thus, he suspects that sea snakes spend a good bit of their lives thirsty, which they seem to withstand quite well. Even with a more than 20% loss of body weight, yellow-bellied sea snakes are fine; just a 12% loss in humans due to dehydration could be lethal. Salt glands, impermeable skin, nasal valves that keep salt water out, and an ability to extract water from feces and possibly from ingested prey may help slow dehydration, he points out.

Additional work by Brischoux and Lillywhite suggests that the overall distribution of sea snakes is influenced by salinity,



Are you thirsty? Harvey Lillywhite has tested hundreds of sea snakes to see if they will drink fresh water.

and, by association, rainfall. With colleagues, they compared the ranges of four lineages of marine snakes—about 75 species in all—to satellite information about the water’s salinity. There tend to be more sea snake species in areas with lower salinity or with higher variability in salinity (a likely indicator of heavy rainfall), the researchers reported this month in *Ecography*.

“The whole idea of sea snakes not being completely adapted to the oceans as we thought they were is a very interesting revelation,” Murphy says. “Even though sea snakes have invaded the oceans, they are still very dependent on fresh water.”

—ELIZABETH PENNISI



Dark knights. The Crusaders who built Poland's grand Malbork Castle also helped drive native animals to extinction.

ogy of conquest elsewhere. "What they're doing is looking at the environmental data to understand how one group imposes itself on an indigenous population," says Mark Brisbane, an archaeologist at Bournemouth University in the United Kingdom who is familiar with the project. "I certainly think it's an innovative approach."

Making war

The crusading movement began in 1095, when Pope Urban II urged European Christians to free the Holy Land from Muslim rule. Warriors, knights, and adventurers from across Europe joined, many motivated by the church's promise that fighting would absolve their sins. The Teutonic Knights, an order of warrior-monks, were among the most powerful players. In 1228, the Teutonic Order turned to easier targets: pagan tribes living in an unruly stretch of northern Europe from Estonia to what is now Poland. These Northern Crusades didn't have the emotional appeal of seizing Jerusalem, but they still offered the hope of eternal salvation—and the chance to carve a new, resource-rich state into the map of Europe.

Although the rest of Europe had converted to Christianity and organized into states and kingdoms by the turn of the millennium, these lands along the Baltic Sea had changed little in 1000 years. "There's no written culture that we're aware of," Pluskowski says. "We're dealing, in effect, with prehistoric societies." There were no real urban centers, just small, sedentary settlements organized on family or tribal lines. People farmed rye, wheat, barley, and millet in small plots, and wild game made up a substantial part of the diet.

Almost immediately after conquering territory, the Northern Crusaders began building some of Europe's most imposing strongholds and towns. The edifices include Malbork Castle in Poland, which is a World Heritage site and one of the world's largest medieval fortresses. In Prussia, Crusaders killed or drove out most of the indigenous people, bringing in German settlers to repopulate the land.

While working at Malbork in 2007, Pluskowski wondered whether the changes he saw in the local fauna could be seen at other Crusader sites. With funding from the European Research Council, he put together

ARCHAEOLOGY

Crusader Crisis: How Conquest Transformed Northern Europe

A novel interdisciplinary project shows how medieval crusaders, with their new way of life, suddenly changed the ecology of northern Europe

Last summer, archaeologists working in the central courtyard of Estonia's Karksi Castle uncovered a 50-centimeter-thick layer of rich black dirt. As the researchers dug deeper, they realized they had discovered the remains of the castle's first garbage pit. Preserved inside was a snapshot of what the first inhabitants of Karksi Castle—dozens of German knights and their servants—had eaten and discarded more than 800 years before.

The pit's wet soil had preserved a wide variety of objects, such as hazelnuts, fish scales, animal bones, and hemp seeds, says archaeologist Heiki Valk of the University of Tartu in Estonia. But what surprised Valk most was what wasn't in the garbage. "The early material is very strange—there's absolutely no local pottery," he says. "The colonists came with a lifestyle that didn't fit the local environment at all. They were a little island, with everyday life just like it was in Germany."

The knights of Karksi Castle were part of the little-known "Northern Crusades," an era of conflict that gripped northeastern Europe between about 1200 and 1400 C.E. when German Crusaders turned their attention from the Holy Land to pagan tribes on the fringe of Europe. Today, a team of researchers is documenting the ecological impact of this military conquest and the coloniza-

tion that followed. The project, one of the first of its kind, combines traditional archaeology with close looks at animal and plant remains, geochemical analysis, and archival research. Similar approaches have been used to look at the impact of colonialism in New Zealand and elsewhere, but the Ecology of Crusading project is particularly ambitious in terms of the number of sites and the use of historical sources.

The Northern Crusades involved not just religious conversion but also often the replacement of entire populations. "This pre-Christian society is conquered by external people who bring their own language and religion, Christianity, with them," says Aleksander Pluskowski, an archaeologist at the University of Reading in the United Kingdom and director of the project (www.ecologyofcrusading.com). "It's sort of an event horizon in northeastern Europe." In large stretches of territory in an area from what is now Estonia to modern Poland, the locals were killed or fled; in others areas, the elites were replaced and the locals slowly assimilated.

New arrivals from Germany brought with them new diets, lifestyles, and subsistence strategies—a process not unlike what happened as pioneers settled the American West. Colleagues say understanding the environmental signal of such a phenomenon can bring a new perspective to the archaeol-

a multinational team of historians, archaeologists, zooarchaeologists, geoarchaeologists, and paleobotanists to look at a dozen sites in five countries.

So far, their work shows that the Crusades brought tremendous ecological and cultural change. “What you have in the Baltic is the abandonment of tribal sites and the appearance of new structures dated to the 13th and 14th centuries,” Pluskowski says. “Castles, churches, towns—it’s the replacement of one culture by another without a smooth transition. It all seems very sudden.”

Fortresses served as bases for further fighting and as centers of trade where knights exported goods such as furs, timber, and grain from their new territories. The invaders had an immediate, dramatic effect on the landscape. “A castle in a landscape is a huge pull for resources,” says Krish Seetah, an archaeologist at Stanford University in Palo Alto, California, and one of the project’s faunal experts.

Some of the changes are obvious in the archaeological record. Quarries show up, dug to meet the need for sprawling castles of stone, brick, and clay. The architecture looks as though entire villages were imported from Germany. Urban centers concentrated organic waste, along with remains of rats and cats.

A lost world

Other changes were more subtle. Ample beaver bones, for instance, show large-scale hunting and trapping for furs. “There’s a distinct intensification in the exploitation of the natural world that continues to this day,” Pluskowski says. Large mammals like wolves and bison never really recovered; aurochs, forerunners to modern cows, were hunted to extinction within a few centuries.

The team’s work also illuminates cultural changes. Written sources like contracts and castle inventories give historians an idea of what was being stored in the castles, such as cows, goats, bison meat, and bags of grain. Analysis of animal bones fleshes out the record further, so to speak. By comparing bones found in digs at local settlements with remains at the newly built Crusader castles, zooarchaeologists can track shifts in diet and culture. For example, dog bones found at one indigenous settlement show evidence of cutting consistent with butchery for meat.

Those practices end abruptly after the Crusades begin. Cattle and other domesticated animals replace the aurochs, roe deer, elk, wild boar, and bison that local people once hunted. “It would seem as though something quite dramatic happens and a prohibition on eating wild dogs comes into effect, along with a reduction in the exploitation of wild animals,” Seetah says. Technology changed, too: Cut marks on bones suggest that the newcomers brought larger, heavier blades for processing meat. The Crusaders also quickly replaced the small local horses with massive warhorses capable of carrying the weight of a fully armored knight into battle.



of the appearance of the Order and the scale of resources required to construct castles and maintain them.”

Colleagues say this is the first time that anyone has taken such a broad look at the environmental impact of a Crusade. Similar work is just beginning in the Holy Land, where Crusader archaeology has traditionally taken a back seat to biblical archaeology, says archaeologist Adrian Boas of the University of Haifa in Israel: “The Crusades in Israel haven’t been studied in depth.”

That may soon change. Boas recently began a long-term project at Israel’s Montfort Castle, once the Teutonic Order’s headquarters in the Holy Land, hoping to



Trash n’ treasure. Medieval refuse at Karksi Castle (left) in Estonia and a horse skeleton at Cēsis Castle in Latvia show Crusaders’ impact.

use similar techniques to explore the Crusaders’ impact. “We’re planning a lot more work in this field,” Boas says. There are already interesting connections—Crusaders apparently brought pigs to the Holy Land, for example.

The project’s approach could also help untangle the impact of colonization in other places. Pluskowski would like to look at Spain, where the Reconquista of the 1400s wrested control away from Muslim rulers, forming a sort of final coda to the Crusades. The Mongol invasions of Hungary and Romania may have also left impressions in the environmental record.

It might even be possible to turn the clock 1000 years further back, to look at how the Northern Crusades compare to the expansion of the Roman Empire. Pluskowski says: “This project pushes us in a direction where we can compare more generally the impact of human colonization.”

—ANDREW CURRY

Andrew Curry is a freelance writer based in Berlin.

REGULATORY SCIENCE

Amid Europe's Food Fights, EFSA Keeps Its Eyes on the Evidence

Europe's food-safety watchdog, celebrating its 10th anniversary this month, wins praise for sticking to the science—even when Europeans prefer not to hear it

PARMA, ITALY—This small town of quiet squares and historic churches in northern Italy has long been associated with food; it is, after all, the home of the world-famous Parma ham and Parmigiano-Reggiano cheese, also known as Parmesan. But today, Parma plays another big role in Europe's kitchens: It hosts the European Food Safety Authority (EFSA), the agency charged with a wide variety of tasks, such as assessing the safety of genetically modified (GM) plants for 500 million Europeans, preventing *Salmonella* outbreaks, and deciding whether probiotic drinks really boost the immune system.

EFSA was founded in 2002 to help set food standards at the European level; earlier this month, 700 scientists attended its anniversary conference to help celebrate. But if European officials had hoped that Parma's calm atmosphere would rub off on EFSA, they were wrong. The agency has found itself at the center of some of the most ferocious food fights in Europe.

EFSA has angered environmental and consumer groups by ruling again and again that genetically modified organisms (GMOs) and artificial sweeteners pose no health risks—advice governments sometimes choose to ignore. It has come under fierce attack from advocacy groups and politicians for not being totally independent from industry—a charge that inquiries have found not entirely baseless. At the same time, EFSA has upset the food and supplement industry by pushing some of the most aggressive evidence-based policies in the world. Starting on 14 December, manufacturers will no longer be able to tout the health effects of thousands of their products on labels and in ads, because EFSA says they have not been proven.

Amid all of these pressures, “somehow they have managed to doggedly stick to the scientific data no matter what was happening,” says nutrition scientist Martijn Katan of Free University Amsterdam. “I think they have succeeded beyond everybody's wildest expectations.” “It can be slow going at times, but they have done very thorough, good science,” says Hans-Georg Joost, a pharmacologist at the German Institute of Human Nutrition in Potsdam.



Home of the ham. EFSA, headed by Catherine Geslain-Lanéelle (*inset*) since 2006, has its headquarters in Parma, Italy.

Speak up, stand up, gang up

EFSA was established in the wake of several European food scandals in the 1990s that had rocked consumer confidence, including “mad cow disease,” or BSE. “We are a child of the BSE crisis,” says Catherine Geslain-Lanéelle, the French food-safety scientist who has headed EFSA since July 2006. E.U. leaders hoped to marshal the best scientists from across Europe to review food risks and present a strong scientific consensus to guide decision-making. “Before the BSE crisis, it was a hodgepodge of politics and science,” says Andreas Hensel, who heads the German Federal Institute for Risk Assessment. “No one was really responsible for the science on a European level.” EFSA has been a particular blessing for smaller member states that lack their own food-safety agency, he says.

But the science hasn't always won out. EFSA prepares scientific opinions for the European Commission, which then drafts decisions based on them, which are approved or rejected by the Standing Committee on

the Food Chain and Animal Health, consisting of representatives of the member states. The final decision thus rests with politicians, and their opinions vary widely. EFSA has so far approved every GMO that it has reviewed; in the Standing Committee, Luxembourg and Austria have voted against EFSA's opinion on GMOs every single time, whereas Sweden, Finland, the Czech Republic, and the Netherlands have never voted against them.

In a talk at the conference here, Anne Glover, chief scientific adviser to the European Commission, urged scientists to “speak up, stand up, and gang up” to defend the science behind EFSA's assessments. “If we do not communicate, it is as if we never did the science,” she told the audience. Countries can vote against GMOs for other reasons, Glover argued, but they cannot reject the evidence just because it's politically inconvenient.

The GMO issue flared up again in September, when a paper in *Food and Chemical Toxicology* concluded that a maize variety called NK603 and low levels of a herbicide could together cause tumors in rats. The study, by French biologist Gilles-Éric Séralini, was widely criticized by scientists for leaving out important information, using rats prone to spontaneous tumors, and looking at too few animals.

Séralini was already well-known at EFSA. In 2007, a panel at the agency looked into his claims that MON 863 maize, which EFSA had given a clean bill of health, was toxic to the liver and kidney in rats. Then, a panel roundly rejected Séralini's analysis, and this time, too, EFSA made short work of his paper. An initial assessment published on 4 October concluded that the study was “of insufficient scientific quality for safety assessments.” (An in-depth analysis was in preparation as *Science* went to press.)

EFSA critics seized on the report as a sign of double standards. Studies of similar quality had been accepted as evidence for the safety of GM food, they argued. But Elisabeth Waigmann, head of EFSA's GMO unit, says there is no comparison, as the studies on which risk assessments are based run hundreds of pages. Séralini's paper was missing too much information to be useful, she says: “We have asked Séralini for the full data, but so far we have not received anything.”

If anything, the issues will only get thornier. Most GM crops approved so far had only a single new trait, usually resis-



tance to a herbicide; in most cases, EFSA's risk analysis was based on comparing those GM crops with the non-GM version of the same variety. But GM plants now being developed often have more than one new gene. "The comparison could then lead to so many differences that need to be followed up individually, that it becomes a question whether this comparative approach is still practical," Waigmann says. EFSA will also have to review GM animals when they are ready—not just those intended for human consumption, but also GM mosquitoes set free to curb the spread of a disease. The agency is still drawing up guidelines for that task.

Claims and conflicts

Conflicts about scientific evidence have also occurred with EFSA's biggest and most ambitious project so far. Starting next month, companies in the European Union will no longer be allowed to advertise food products with unsubstantiated promises about their effect on human health. Since 2008, EFSA panels have assessed thousands of such health claims, including cranberry juice supposedly reducing the risk of urinary tract infections and a type of chocolate that its producer says "helps children grow" (*Science*, 5 March 2010, p. 1189). Of more than 3000 claims, only some 200 were deemed sufficiently proven.

The food industry has protested vigorously. "EFSA's methodology is almost identical to that for assessing medicine. It is based on randomized controlled trials, and that is not feasible for food," says Patrick Coppens of the European Responsible Nutrition Alliance, an industry group. Such trials would be far too expensive, and the rules could stifle innovation, he warns.

Industry representatives have also pointed out that there is no clear consensus on what a health claim is. Some national authorities have hinted that they will no longer accept the term "probiotics" in marketing, for instance, because the term itself suggests a health benefit; other countries say it's just a product category. Some people have joked that German candy manufacturer Haribo may have to submit evidence to prove its slogan that "Haribo makes children happy!"

"Industry was surprised how much science was needed," says Juliane Kleiner, head of EFSA's nutrition unit. "But these are no outrageous criteria." Katan says the mass rejection of claims reflects the fact that the food industry has been unable to produce food that makes a real difference for health. By being so rigorous, EFSA has set a new standard, he



Seeds of conflict. Activists have taken aim at EFSA's assessments of GM crops.

says: "The FDA is doing an excellent job on the safety, but as to health claims, the world is now looking to Europe."

The process has produced new problems, however. For instance, the European Commission put EFSA's evaluation of botanical products on hold because it could lead to a paradox: Health claims of herbal foods could be dismissed on scientific grounds, but the same substances could still be sold as medicine because in many European countries, they can be marketed on so-called traditional

May, when Diána Bánáti, chair of the management board of EFSA, had to resign effective immediately because she had accepted a position at the International Life Sciences Institute, a nonprofit science organization based in Washington, D.C., and funded among others by Coca-Cola, McDonald's, and Monsanto. Members of the European Parliament have pounded on the issue, and the Parliament temporarily deferred approval of EFSA's 2010 budget report to put pressure on the agency.

A long-delayed report by the European Court of Auditors, issued in October, showed that the accusations had some basis in fact. The report—which also looked at three other agencies—concluded that EFSA's management of conflict-of-interest situations was not adequate. It said there were either no clear criteria for assessing declarations of interest, or that they were not applied consistently.

EFSA responded that it had addressed several of the critical points in the meantime with a new independence policy adopted in December 2011—the review finished in October 2011—and that it had assessed 8000 declarations of interest in 2011 and excluded experts totally or partially from EFSA activities on 356 occasions. Nina Holland of CEO agrees that there have been some improvements, but several forms of conflict of interest are still allowed, she argues.

Geslain-Lanéelle says that dismissing everyone with any ties to industry would make it impossible to find good experts. If



Not convinced. EFSA rejected supposed health benefits of rapeseed oil, cranberry juice, coffee, and chocolate, along with thousands of other claims.

use grounds with no need to prove efficacy. How this issue will be resolved is still unclear.

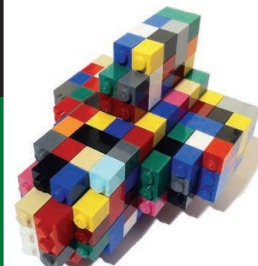
But EFSA's long-term credibility will depend not just on the rigor of its reviews, but also on the trust it enjoys from the European public—and on that front, it has fared less well. In February, a report by two campaign groups, Corporate Europe Observatory (CEO) and Earth Open Source, concluded that "[m]any EFSA panel members have ties with biotech, food, or pesticide companies." EFSA's rules allowed blatant conflicts of interest to persist, the groups said.

EFSA dismissed the report as biased and unfounded, but the criticism only increased in

someone has worked with industry, that person will not be allowed to work with EFSA on the same subject, she says. Moreover, decisions are made by panels that usually have 21 members. "Even if you had one or two experts with a conflict of interest, they would have to convince another 20 experts. And believe me, they are not easy to convince."

Still, Geslain-Lanéelle acknowledges that EFSA needs to work on building trust. But the fact that science is now center stage in the decision-making process in the E.U. is new, she says, and reason to celebrate. "Don't forget: We are only 10 years old."

—KAI KUPFERSCHMIDT



LETTERS

edited by Jennifer Sills

The K Computer: User-Friendly

THE NEWS & ANALYSIS ARTICLE "UTILITY SACRIFICED FOR SPEED, supercomputer critics say" (D. Normile, 5 October, p. 26) discussed our project, the Japanese 10-petaflops supercomputer (or "K computer"). Some criticisms reported in the article were based on misunderstandings, which I would like to clarify.

Contrary to the title "utility sacrificed for speed," I am confident that the K computer is the most user-friendly among the world's top-ranking supercomputers. In designing it, we focused not only on peak performance but also on sustained performance on real applications in a wide range of science and engineering fields. For example, the memory and network bandwidth per performance (1), which are key factors for application efficiency, are much better than those of IBM Sequoia (BlueGene/Q) at Lawrence Livermore National Laboratory (2) or Cray Titan at Oak Ridge National Laboratory (3, 4). We have also adopted the widely used standard programming model (MPI/openMP) as in BlueGene/Q. The K computer demonstrated an extraordinary level of stability for one of the world's largest-scale systems; the overall system comprised of 88,128 CPUs ran without a single failure for 29.5 hours. All these features (high bandwidth, standard software environment, and high reliability) require extra hardware and software costs, which decrease the peak performance possible for a given cost. Thus, we are proud to say that in reality, we sacrificed speed for utility in the K computer design.

In the article, Jun Makino implied that we did not explore the design sufficiently. The truth is that

we accepted proposals on architecture from academia and industry in the design process. In the selection of benchmark applications, we paid special attention to the parallelizability (i.e., the marginal number of processors that minimize an execution time of an application) of the algorithms we used, which is strongly related to optimal supercomputer architecture. Performance was never the only criterion: Some proposals were retracted, and others judged as infeasible due to their weak developmental systems.

I am confident that we explored various architectural possibilities, taking into consideration their relations to algorithms.

Takafumi Matsui said that the project was designed just to serve the computer industry. This is not the case. Of the total project cost of \$1.4 billion, \$500 million was devoted to software development for life sciences and nanoscience, in addition to buildings and facilities. We recognize that the project's main goal was the development of the supercomputer, with the goal of broad application in science and engineering. Is this a bad thing? It would be if no one wanted to use our computer. The good news, as the article noted, is that many users have applied to use the K computer. Its true value will soon be proved.

KIMIHIKO HIRAO

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Supercomputer. Japan's K computer contains 864 water-cooled cabinets.

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The Final End of the Final Frontier?

THE ELECTIONS HAVE NOW PASSED, BUT the planetary exploration effort of the United States remains anything but settled. Deadlocks remain, with a continuing resolution in place through next March, no relief in sight for sequestration to be averted, and the \$309 million cut in planetary exploration for the proposed fiscal year 2013 (FY2013) budget already in place. The proposed FY2014

level in the President's FY2013 budget release mandates an additional cut of \$58.6 million on top of the already precipitous drop from the FY2012 budget numbers to those of FY2013. The potential for planetary exploration is a drop of about \$370 million in 2 years, a decrease of one-quarter of the total planetary exploration program, with further cuts slated for FY2015 (1).

NASA currently accounts for a near-historical low of 0.46% of the U.S. government budget (2). After 50 years of robotic planetary exploration, we know the high cost

of space exploration, robotic and human. Doing worthwhile things is not cheap, but slashing budgets in opposition to long-term national goals is a policy error.

These NASA dollars enable activities in space but are spent on the ground. NASA funding has led to advances in electronics, robotics, and aeronautics. Space-based, scientific investigation of Earth's resources and climate are of increasing importance. And of course, the money goes toward salaries for high-wage, high-skill jobs. NASA has time and again delivered the best value

per dollar back to the American taxpayer, providing new knowledge and understanding that we can all be proud of as a joint American undertaking. The return on investment, in our current quality of life, in future generations, and in the achievements of our country has been immense.

We urge the Administration, the White House, the Office of Management and Budget, and Office of Science and Technology Policy, along with both houses of Congress and both political parties, to deal with the current imbroglio and work together to continue the nation's investment in our successful program of solar system exploration. Even in tight budget times, the return is well worth the price.

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Animal Welfare Development in China

THE THIRD CHINESE VETERINARY CONFERENCE was held in Suzhou, China, from 28 to 30 October (1). For the first time at a Chinese national conference, a forum on Animal Welfare Development was included on the schedule. The addition of this topic reflects the growing recognition of the importance of animal welfare issues among the Chinese academic community. At the forum, Chinese veterinarians and international experts agreed that improving animal welfare in China will require the development and enforcement of a national code of animal welfare, and that reference texts and educational materials in Chinese will be needed to standardize teaching and to make the issue broadly accessible.

Animal protection and welfare issues are also gaining increasing attention in mainstream Chinese society. Take, for example, the recent public support for banning the practice of medicinal bear farming, which involves repeatedly extracting bile from live, captive animals (2). Popular figures are also promot-

ing animal welfare issues: For example, the legendary basketball star Yao Ming is the face of a new WildAid campaign, which is combating the ivory trade by targeting potential consumers (3). A suite of other animal advocacy nongovernmental organizations (NGOs) such as the World Society for the Protection of Animals (WSPA) (4), Animals Asia (5), and the International Fund for Animal Welfare (IFAW) (6) also operate within China. Despite this growing awareness, however, China has a long way to go in developing and implementing animal welfare standards at an international level. We offer the following suggestions to facilitate this process.

China must develop and enact national animal welfare legislation and policies. Although related legislation has been in preparation for years, there are still only fragmentary professional requirements and no national laws dealing directly with animal welfare. The legislation has been delayed by a range of issues, including the need for measurable animal welfare criteria, and the potential for conflict between policy supporters and commercial interests. These issues must be resolved and the legislation process

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completed as a matter of urgency, in order to provide a stable basis for the improvement of animal welfare across China.

Effective implementation of such legislation will require the establishment of government institutions as a platform for the assessment and monitoring of the welfare of farmed, captive, and pet animals across China. Such institutions will require the participation and cooperation of relevant government departments, the academic community, commercial interests, and consumers. Together, these stakeholders can develop and enforce effective and sustainable animal welfare procedures and standards.

To accurately convey the concepts of animal welfare and the need for animal welfare standards, community education will be critical. These activities should be targeted especially to professionals who work closely with animals—veterinarians, animal keepers, and researchers—who, by changing their own behaviors, can influence the behavior of the communities in which they work. Relevant educational materials should also be distributed through collaborations with schools and community groups in an effort

to educate and influence the largest possible cross section of society.

Animal welfare research in China has been limited, in large part due to a lack of funding. This is in turn partly due to the lack of enforced animal welfare standards for animals-related industries, and also to the perception that introducing animal welfare standards can only negatively affect production and profits. Relevant research should therefore be supported and encouraged. Collaborations among Chinese researchers, NGOs, and the international academic communities will result in the incorporation of the most effective international policies, industry standards, and research into the development of Chinese education programs and standards.

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CORRECTIONS AND CLARIFICATIONS

News & Analysis: "Disappointing results blunt hopes for malaria vaccine" by G. Vogel (16 November, p. 871). The byline should have stated: "with reporting from Leslie Roberts." The byline has been corrected in the HTML and PDF versions online.

Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the past 3 months or matters of general interest. Letters are not acknowledged upon receipt. Whether published in full or in part, Letters are subject to editing for clarity and space. Letters submitted, published, or posted elsewhere, in print or online, will be disqualified. To submit a Letter, go to www.submit2science.org.

HISTORY OF SCIENCE

The Collective Behind the Genius

Jon Agar

A few years ago Research!America polled Americans to see who could name a living scientist. The most frequently named was the British theoretical physicist Stephen Hawking, known to his peers for his work on the large-scale structure of space-time and the nature of black holes. Millions have read his best-selling popularization of cosmology, *A Brief History of Time* (1). And there are other measures of Hawking's status as the most famous living scientist. No other nonfictional scientist, for example, has appeared so many times (four) in *The Simpsons*.

This esteem derives from a contrast. Since 1963, Hawking has lived with amyotrophic lateral sclerosis, known also as Lou Gehrig's disease. His muscles have atrophied, leaving him dependent on a wheelchair. In 1985, he contracted pneumonia, the complications of which led to a tracheotomy and the loss of his natural voice. The apparent contrast between Hawking's frail Earth-bound body and his powerful, cosmos-ranging mind is central to his public persona and is the source of his immense fame.

Disease has made Hawking different, if not uniquely so, from other researchers. But the separation of body and mind is a familiar stereotype in the representation of modern scientists. We can go back, for example, to William Wordsworth's lines on Isaac Newton in *The Prelude*: "The antechapel where the statue stood/Of Newton with his prism and silent face/The marble index of a mind for ever/Voyaging through strange seas of Thought, alone." Scientists, so this modern myth goes, are idealized as disembodied minds, needing only thought and rational methods. They are "brainiacs."

Media depictions of Hawking, in reviews, biographies, and documentaries, follow this mythology: "Physicist Stephen Hawking is confined to a wheelchair, a virtual prisoner in his own body, but his intel-

lect carries him to the far reaches of the universe" (2). "Where his feet could not go, his mind would soar" (3). Hawking's discoveries are seen as the triumphant achievements of a singular, individual mind.

In *Hawking Incorporated*, the sociologist Hélène Mialet shows, persuasively, that this view is wrong. Her methodological inspirations are ethnography and actor-network theory. Ethnography teaches her to conduct fieldwork, to follow her subjects around and watch, listen, and learn how they make sense of their world. Actor-network theory holds that if we want to analyze these worlds, we should pay attention to the assemblages of entities, human and nonhuman, that define

them. So in the book we can follow Mialet as she examines the networks that center on Hawking, from personal assistants and student helpers, to fellow theoretical physicists, and even to the film-makers, sculptors, and archivists who contribute to making his public image.

Mialet's big idea is the "collective body." This idea is best seen at play in two wonderfully rich chapters, one on Hawking's workplace and the other on how his students help write scholarly papers. Only by training human assistants and connecting to special, mediating machines can Hawking work at all. He has a voice synthesizer and a small computer mounted on his wheelchair. Using what

very limited movement he possesses, including fingers and face twitches, he deploys programs such as EZ Keys and Equalizer to select words. The process is painfully slow, even though Hawking is a skillful and experienced user. Hawking's voice comes from a 1986 synthesizer, made by the now-defunct company Speech Plus. He so identifies with its distinctive intona-

tions that he has, until very recently, refused upgrades. The voice is his, despite its American accent.

Hawking is surrounded by assistants, including a personal assistant (who serves as a key gatekeeper), a nurse, and graduate physicists. They interpret his gestures, moods, and instructions. His house—"the most wired home imaginable"—also forms part of this wider network. "[W]hen Stephen wants his nurse—and his nurse is usually in her room in the back of the house—... he sends an environmental control signal that makes her door open." It's a network activated by Hawking. "Contrary to what the public thinks—that is, that Hawking is pure mind—the role of his flesh-and-blood body is central.... A twitch of an eyebrow or a smile makes the collective function, a collective that is itself an extension of his body."

It is the collective body that writes Hawking's scientific papers. He generally has four graduate assistants, usually drawn from those who excel at Cambridge University's Part III Certificate of Advanced Study. (I did this paper myself; I did not excel.) Hawking seeds ideas for projects; the students then work on the lengthy calculations and write up papers. "When the paper is finally published," writes Mialet, "only Hawking's name will appear on it." She stresses that there is nothing unusual here. Rather, the practice is an indication of how theoretical physics is intensely collaborative but also hierarchical, quite unlike the public image of science being an achievement of individuals alone.

Hawking Incorporated will draw readers because of the extraordinary fame of its subject. However, it is most valuable because its case study identifies exaggerated but important features of ordinary science in practice. Hawking "is not different from other scientists because he is disabled," concludes Mialet, "rather, it is because he is disabled that he offers us the possibility of seeing how scientists work."

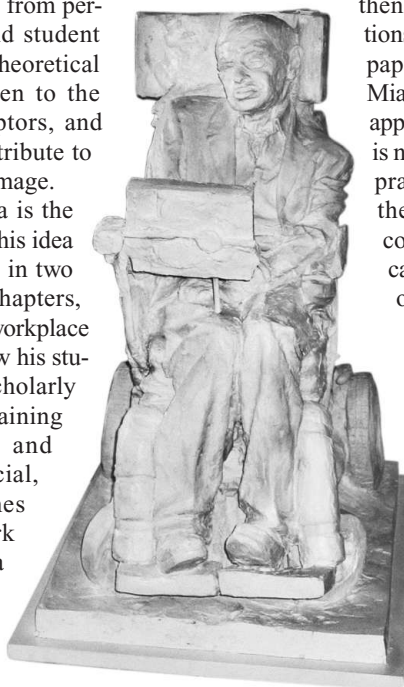
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Hawking Incorporated
Stephen Hawking and
the Anthropology of the
Knowing Subject

by Hélène Mialet

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Eve Shepherd's Hawking statue.

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From Acid Rain to Climate Change

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The Convention on Long-Range Transboundary Air Pollution (CLRTAP) under the United Nations Economic Commission for Europe (UNECE) was established in 1979 to control damage to ecosystems and cultural heritage from acid rain, initially in Europe (1). Extended by eight protocols, most recently the Gothenburg Protocol (GP) signed in 1999, it has been key for developing cross-border air pollution control strategies over the UNECE region, which includes the United States and Canada. We describe how recent amendments to the GP reflect improved scientific knowledge on pollution, environmental relations, and links between regional air pollution and global climate change.

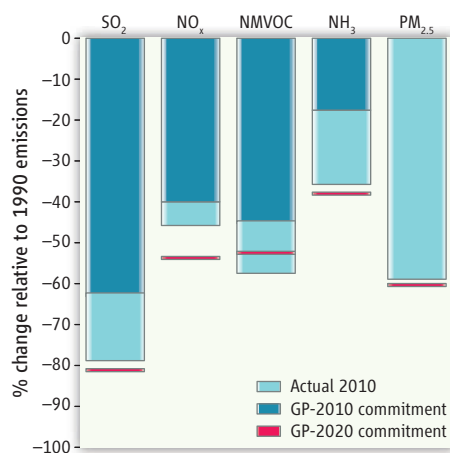
Policy Lessons from CLRTAP

Substantial emissions reductions have been achieved under CLRTAP (see the chart). Air quality has improved, and deposition of acidifying (fig. S1) and eutrophying (fig. S2) compounds in excess of critical loads (2, 3) has been widely reduced. The largest reductions can be seen for sulfur dioxide: Since 1990, several European countries have reduced emissions by close to 80%. Sulfur deposition, once the main cause of the acidification of lakes and soils, has markedly diminished. Although emission reductions were, to a large extent, achieved by cost-effective measures proposed by science, they were also helped by autonomous developments, such as the transition of Eastern European economies (4).

Much of the success of CLRTAP in integrating science and policy is because scientific results, assessments, and technological solutions form an integral part of the agendas of negotiating meetings. Such meetings typically start with an update of the available

science and end with further requests to scientists. Scientists are present in negotiation meetings, and policy-makers participate in scientific meetings and thus can make sure that the science remains focused on the needs of the policy process.

Science played a major role in establishing CLRTAP, substantiated through the European Monitoring and Evaluation Programme (EMEP) and the Working Group on Effects (WGE). In the early stages, the main



Emission reductions and commitments for 2020 under the revised GP. These commitments enter into force upon ratification of the revised protocol by each signatory. All percentage changes are relative to 1990 emissions. Targets of the original GP for 2010 are also shown. GP commitments for nonmethane volatile organic compounds (NMVOCs) for 2020 are above actual emissions for 2010, which were low because of the economic situation in Europe and the related reduction in industrial output and construction. See table S2 for details.

focus was on understanding the causes and effects of the problem and its transboundary nature. A basic building block was quantifying the relations between sources of emissions and pollutant concentrations and/or deposition across Europe, with the aid of atmospheric dispersion models. These relations demonstrated clearly that air pollution was a problem that needed to be addressed at a continental scale.

Further achievements of CLRTAP were the development and consistent application of the critical loads concept, as well as integrated assessment modeling, through which

Updated air pollution science and policies address human health, ecosystem effects, and climate change in Europe.

environmental objectives were used to calculate an economically efficient distribution of effort between countries. The science-policy dialogue became intense in the 1990s, in particular through the Task Force on Integrated Assessment Modelling (TFIAM) and the Working Group on Strategies and Review (WGSR). It was the science that convinced policy-makers that a focus on individual pollutants was leading to suboptimal solutions and that a multipollutant, multieffect approach would be more cost-effective.

The GP, which entered into force in 2005, marked a new approach, scientifically supported and economically justified. Compared with previous international commitments on improving air quality, which contained flat rate reductions for separate pollutants, this effects-based approach identified an optimal allocation of targets among countries to reduce several damaging pollutants simultaneously, leading to considerably lower costs. Each country agreed on emission ceilings to be met for the key pollutants, while retaining some flexibility in how these were to be attained (5). The GP, with its target year 2010, has formed the basis for both international (e.g., European Union) and national policies. Most countries have been able to fulfill commitments in terms of emission reductions with corresponding improvements in air quality.

Revising the GP

The Parties to the Protocol agreed to substantive amendments to the GP in May 2012 (6). For the first time in a multilateral environmental agreement, specific account was taken of the adverse effects of particulate matter (PM) on health. The revision includes commitments for emission reductions of primary particles with an aerodynamic diameter of <2.5 μm (PM_{2.5}). Also, the revised GP reflects links between regional air pollution and global climate change by including black carbon (BC). Like tropospheric ozone, which was already covered by the original GP, BC is a short-lived climate-forcing pollutant (7). The amendments also include commitments to further reduce by 2020 emissions originally covered by the GP (sulfur dioxide, nitrogen oxides, ammonia, and volatile organic compounds) and to maintain these reductions thereafter.

Russia, Belarus, Canada, and other coun-

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tries that are not parties to the original GP participated in negotiating the amendments. The text opens avenues for these and other countries to accede to the GP, which increases the spatial coverage of agreed emission-abatement measures and the political weight of the CLRTAP. Wider coverage will also facilitate scientific development and capacity-building by integrating countries with limited experience in the design and implementation of emission control policies into an established science-policy framework.

Scientific findings have shaped and informed policy revisions by, for example, assessments of relations between air pollution abatement and impacts on human health and the environment. Illustrations of environmental impacts of different policy scenarios using new indicators (e.g., updated critical levels and loads, or economic impacts from ozone on crops) have influenced explicit inclusion of PM to reduce health effects. Inevitably, financial and political realities constrained negotiations with respect to scientific objectives. However, integration of science and policy into an accepted model that assesses technological, economical, ecological, and public health issues enabled new countries to agree on emission reductions, even in times of economic austerity.

Parties revised their “baselines” after recent changes in economic projections; thus, most parties’ commitments to the revised protocol fall short of the reductions previously projected for the year 2020 (8) that were intended to take into account current legislation and policies on energy and environment already in the pipeline. The revised GP does not include mandatory abatement measures for small combustion installations and mobile machinery nor additional mandatory measures reducing ammonia emissions from agriculture. Despite the limited ambitions in the GP amendments, substantial improvements in air quality are expected over coming years. Yet, these measures will not be enough to meet air-quality targets, such as critical loads and World Health Organization guidelines, to protect the environment and public health. Although the agreed emission levels imply that by 2020 only 4% of European ecosystems will be at risk of acidification, 42% of European terrestrial ecosystems will remain at risk from nitrogen eutrophication and consequent biodiversity loss (9) (figs. S1 and S2 and table S1). The average loss in human life expectancy attributable to exposure to fine PM will decline from 7.4 months in 2005 to 4.4 months in 2020 (8).

Even if hemispheric background concentrations of ozone have increased, control

of ozone precursor emissions according to amended GP targets is expected to further reduce peak ozone concentrations and their effects on human health and agriculture. The loss in wheat production due to ozone is expected to decrease from 27 million metric tons in 2000 to 16.5 million metric tons in 2020 (4), corresponding to a total value of €1.96 billion (U.S. \$2.49 billion) saved. Despite this, effects of ozone on food security and carbon sequestration will remain an issue (10).

Challenges After the GP Revision

After the GP revision, CLRTAP faces additional challenges. The parties recently agreed on a long-term strategy (11), emphasizing scientific guidance and further development based on minimizing adverse environmental and health effects most efficiently. It further emphasizes how CLRTAP must assess multiple interactions of air pollution effects in relation to climate change, health, and biodiversity. Although economic assessment of costs and benefits has long been performed under CLRTAP (12), recognition of the scale and variety of benefits to society from environmental improvements under CLRTAP needs to be factored into policy-making.

Air and climate cannot be tackled independently of each other; nevertheless, linking air pollution and climate change policies more closely remains a challenge. This includes short-lived climate forcing and environmental effects of BC and tropospheric ozone (13) and the effect of nitrous oxide on stratospheric ozone depletion (14).

Another challenge is to account for intercontinental transport of atmospheric pollutants, for more cost-effective policy options. Both increased hemispheric background levels of ozone and transport of many persistent organic compounds and mercury between continents and into the Arctic have been thoroughly investigated (15). These problems need activities both within and outside CLRTAP. CLRTAP promotes collaborative efforts on a multiregional or global scale, such as through the Task Force on Hemispheric Air Pollution, in which many Asian scientists are involved, the UN Environment Program (UNEP), and the Global Air Pollution Forum.

The European Nitrogen Assessment (16) established that an integrated approach to manage nitrogen fluxes and control emissions is lacking. Because much of the problem is related to atmospheric emissions, the CLRTAP requested its Task Force on Reactive Nitrogen to develop an integrated policy approach for nitrogen. Inclusion of national nitrogen budgets in the amendments to

the GP is a step in that direction, and the Task Force is developing partnerships with UNEP and the Organization for Economic Cooperation and Development, as well as the biodiversity and water conventions, on improving nitrogen use efficiency.

Close collaboration and interaction of science with policy plays an important role in the CLRTAP. In this regard, it differs from the UN Framework Convention on Climate Change, as the science-policy interface under the CLRTAP is embedded in its organizational structure (17). The organization of scientific bodies (EMEP, WGE, and their subsidiary bodies) within CLRTAP, as well as the work done bridging science and policy within the TFIAM and the WGSF, make the CLRTAP a unique model worth wider consideration within different international policy forums.

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Supplementary Materials

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MEDICINE

iPSC Disease Modeling

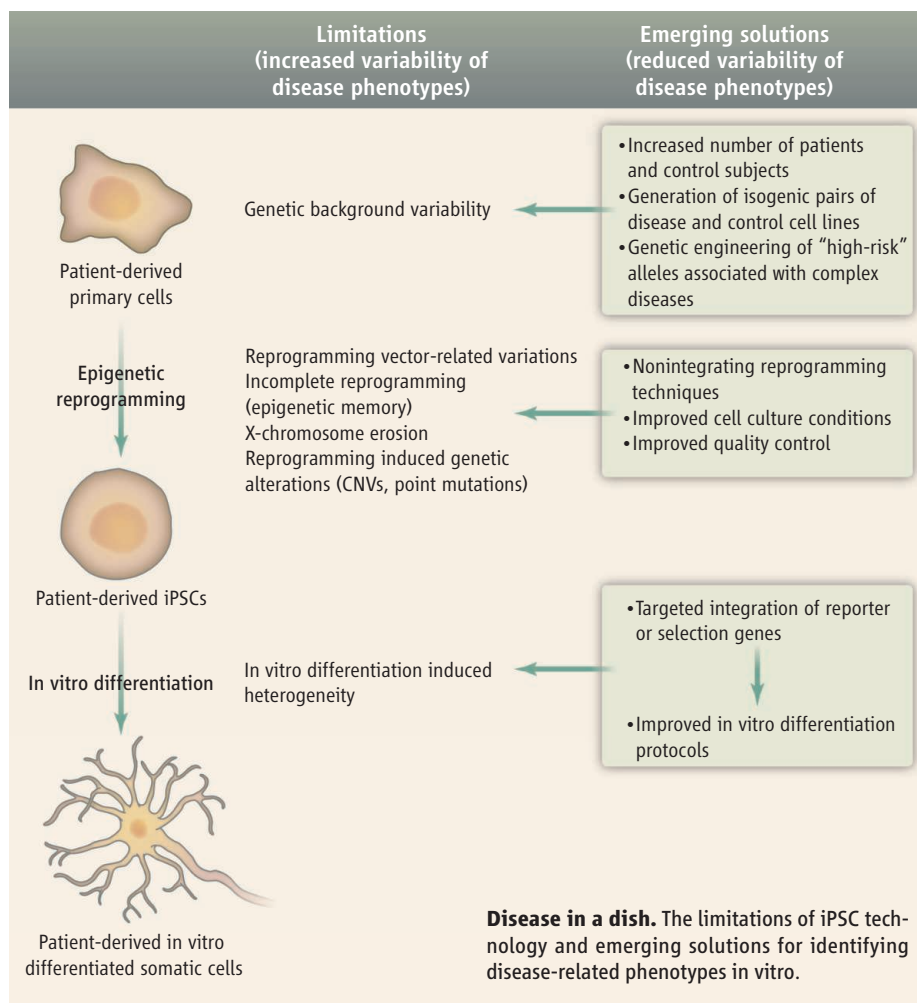
Frank Soldner¹ and Rudolf Jaenisch^{1,2}

Induced pluripotent stem cell (iPSC) technology has provided previously unanticipated possibilities to model human disease in the culture dish. Reprogramming somatic cells from patients into an embryonic stem cell–like state (1) followed by differentiation into disease-relevant cell types can generate an unlim-

than studies using genetically well-defined model systems. Here we describe some of these limitations, and also present some solutions for ensuring that iPSC technology lives up to at least some of its promise.

Individual iPSC lines, independent of disease status, display highly variable biological properties (3–5). This makes their

Induced pluripotent stem cell technology has great potential to model human diseases, but faces many challenges.



ited source of human tissue carrying the genetic variations that caused or facilitated disease development (2). Yet, despite the excitement over this "disease-in-a-dish" approach, studying genetic disorders in patient-derived cells faces more challenges

propensity to differentiate into specific functional cell types unpredictable, thereby limiting their value for studying disease-specific phenotypes. The many reasons for these cell-to-cell differences can be classified into three categories (see the figure): cellular changes resulting from the reprogramming process; culture-induced differences due to the lack of robust differentiation protocols; and differences in genetic background.

Cellular changes caused by reprogramming are especially problematic if the vectors integrate into the host genome. This can result in disruption or dysregulation of nearby genes and often entails residual expression of the reprogramming transgenes (6). Additional complications are caused by incomplete epigenetic reprogramming (7) influenced in part by the stoichiometry of the reprogramming factors and specific culture conditions (8, 9), transcriptional derepression of genes on the inactivated X chromosome (10), and genetic alterations such as point mutations and copy number variations (CNVs) (11, 12). However, more advanced nonintegrating reprogramming technologies such as plasmid or mRNA transfection and protein transduction, improved growth conditions, and more stringent quality control should adequately address these limitations (1, 13).

A major reason for cell-to-cell variability is the lack of robust in vitro differentiation protocols. Most protocols rely on growth factor signals and regulators that play essential roles at specific stages of normal embryonic development. Although fairly efficient in generating some cells of interest, these protocols typically produce a mixture of diverse cell types, which is a crucial limitation when the goal is to create highly controlled conditions. One widely discussed improvement is to introduce reporter or selection genes under the control of lineage- or cell-type-specific promoters (14), thereby allowing the identification, selection, and quantification of specific cell types.

Genetic background variations present a particular impediment to the disease-in-a-dish approach owing to the uncontrolled impact of genetic modifier loci. Epistatic effects from genetic background are observed even in the most prevalent monogenetic disorders causing, for example, variable age of onset and/or disease progression. An individual typically differs from the reference genome at several thousand sites in protein-coding genes, hundreds of which are predicted to alter protein function with many implicated in inherited disorders (15–17). Likewise, there is a major influence of genetic variants on gene expression differences across individuals (18). Therefore, the comparison of patient-derived

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iPSCs to cell lines from healthy donors that share no or only partial genetic background poses considerable risks for interpreting a supposedly disease-related phenotype. This becomes particularly relevant for most late-onset diseases that typically show slow progression of pathophysiological changes and therefore are expected to display only subtle changes in vitro, which may not fully manifest during a short study period.

Recent advances in gene-editing technologies enable the targeted modification of human cells for gene disruptions, genetic repair, or insertion of reporter genes (14). The ability to modify single base pairs, thereby seamlessly correcting or introducing disease-causing mutations in human pluripotent stem cells, allows the creation of genetically controlled experimental

model systems in which the disease-causing genetic variation is the sole experimental variable (19–21). This could substantially simplify the analysis of the interaction between these genomic variants and disease phenotypes, thereby revealing new insights into the pathophysiology of monogenic and complex diseases.

The combination of iPSC, gene editing, and genome-wide technologies gives us the opportunity to systematically and faithfully model human disease in relevant human cell types. Acknowledging the inherent limitations and subjecting iPSC technology to the same rigor that has become standard in other model systems will make it an indispensable tool for biomedical research.

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NEUROSCIENCE

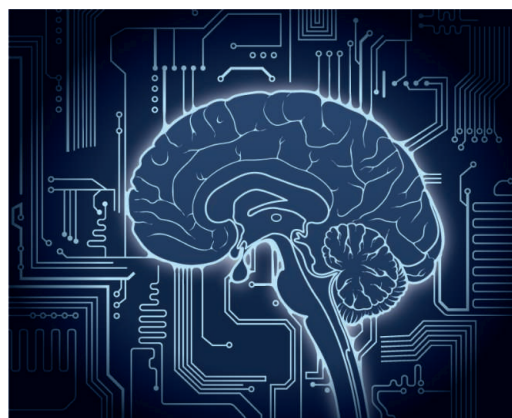
Building the Human Brain

Christian K. Machens

The human brain is exceedingly complex and studying it encompasses gathering information across a range of levels, from molecular processes to behavior. The sheer breadth of this undertaking has perhaps led to an increased specialization of brain research and a concomitant fragmentation of our knowledge. A potential solution is to integrate all of this knowledge into a coherent simulation of the brain (1). However, simply “building” a brain from the bottom up by replicating its parts, connections, and organization fails to capture its essential function—complex behavior (2). Instead, just as engineers can only construct cars and computers because they know how they work, we will only be able to construct a brain if we know how it works—that is, if we understand the computations that are carried out in individual brain areas, and how these computations are implemented on the level of neural networks. On page 1202 of this issue, Eliasmith *et al.* (3) make headway toward this benchmark by presenting just such a large-scale computational model of the human brain that can simulate a variety of complex behaviors.

The model of Eliasmith *et al.*, called the Semantic Pointer Architecture Unified

Network (or “Spaun”), can observe visual images and indicate its responses with a physical model of an arm. The authors show that Spaun can perform eight quite diverse tasks, all of which involve the presentation of various images (mostly numbers) and subsequent motor responses (numbers drawn with



the arm). The tasks range from simple perceptual tasks (image recognition) to working memory tasks (sequence recall) and reinforcement learning tasks (a gambling task) to complex cognitive tasks [aspects of an intelligence quotient (IQ) test]. Spaun performs all of these tasks based on the activity of 2.5 million simulated neurons that are organized into subsystems resembling different brain areas, and wired up

to provide the necessary functionality.

The incoming visual image is first “compressed” so that any irrelevant or redundant image parts are eliminated. Compression is achieved through a so-called hierarchy of restricted Boltzmann machines (in a feed-forward neural network) in which successive layers extract more and more complex features of the visual input (4). The authors associate these layers with areas of the brain that comprise the ventral visual stream (the primary and secondary visual cortex, the extrastriate cortex, and the inferior temporal cortex). On the motor side, Spaun turns a simple, internally generated command signal (for example, how to draw the number six) into a complex arm movement that consists of many elementary motions. The relevant computations are based on optimal control theory (5) and are associated with supplementary motor areas and the primary motor cortex.

This combined compression and expansion of information solves the “curse of dimensionality” in dealing with the environment—the problem of handling an incredibly vast amount of sensory information and choosing from an incredibly vast amount of possible motor outputs.

The actual cognitive machinery of Spaun consists of two intertwined components: a working memory system [prefrontal cortex

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(PFC)] and an action selection system (basal ganglia and thalamus). The latter controls the current brain state and is partly inspired by the theory of reinforcement learning and a contemporary model of the basal ganglia (6). Spaun's working memory is based on an innovative amalgam of neural integrators taken from (computational) neuroscience (7), and so-called convolution memories, taken from (mathematical) psychology (8). The neural integrators provide Spaun with a network mechanism to store information, whereas the convolution memories provide a memory-efficient algorithm that allows Spaun to bind newly arriving information with already stored information. Spaun thereby replicates behavioral effects such as primacy and recency: a tendency to remember the first and last item in a list better than any others.

An additional working memory system is used to automatically infer relations between past and present stimuli. This automatic inference amounts to a simple case of syntactic generalization and is made possible by the way Spaun handles the representation of numbers. These representations provide a direct link to the symbolic computations common to the connectionist (and computer science) literature. Spaun uses these computations to pass some basic aspects of an IQ test.

The systems in Spaun allocated to the PFC bridge the gap between abstract, symbolic computations and the activity of single neurons. Particularly with respect to the convolution memories, Eliasmith *et al.* make some interesting predictions of how firing rates of neurons (the average number of electric impulses or "spikes" per unit

time) should evolve during sequential working memory tasks. These predictions are worth investigating experimentally, especially because sequential working memory tasks have been employed in monkey electrophysiology, so that the right type of data may already be known.

In each of Spaun's areas or modules, the actual information is processed through populations of spiking (active) neurons. The link between the high-level computations performed by the networks and the low-level computations performed by individual neurons is constructed by means of the "neural engineering framework" (9), which specifies how to implement arbitrary mathematical vector operations in spiking neural networks. The framework assumes that information is read out linearly from neural firing rates and is transformed through the nonlinearities of neural activation functions. Consequently, the information processed by each area is distributed across neurons, and thereby roughly matches some of the known electrophysiological features of the areas, such as the diverse tuning of neural firing rates to sensory stimuli or motor outputs.

Given the scope of the model engineered by Eliasmith *et al.*, it is not surprising that many aspects of Spaun deviate from real brains. For instance, the spiking activity within many areas differs in several aspects (including basic statistical aspects) from that measured in real brains. To what extent this problem can be remedied in future work, or to what extent these discrepancies point toward fundamentally different computations in the brain, is currently unclear. Spaun's principal short-

coming is that it is essentially hard-wired and cannot learn any new tasks. Its architecture is quite flexible and not bound to particular tasks, and several parts of Spaun are learned (such as the visual hierarchy or the values of actions). However, learning in its broadest sense, such as learning completely new tasks, is one of the issues that the authors have deliberately—and perhaps wisely—side-stepped. Indeed, just as Spaun falls short on this point, so does our understanding of the brain. By assembling a large amount of brain know-how into one model, Eliasmith *et al.* have provided a coherent theory of how the brain works (with the exception of learning). To paraphrase the statistician George Box, their model is likely to be wrong, but it is certainly useful. Moreover, the authors have provided an opening gambit for top-down approaches to large-scale simulations of the brain. Spaun levels the playing field by setting a new goal and a new benchmark for such simulations: to not simply incorporate the largest number of neurons or the greatest amount of detail, but to reproduce the largest amount of functionality and behavior.

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ECOLOGY

Integrating the Captive and the Wild

Kent H. Redford,¹ Deborah B. Jensen,² James J. Breheny³

Conservation biology was founded with a focus on the plight of species by a group of scientists that included representatives of the zoo and botanical garden communities (1). In the decades since then, zoos have sought to minimize their impact on populations of wild animals and to contribute to field conservation while main-

taining genetically and demographically sustainable captive populations and using these animals to help educate the public about conservation. In a recent article, Lacy argued that zoos are not achieving genetic sustainability for target animal populations (2). However, zoos have contributed a set of approaches to species management that are being integrated with those from field conservation to create hybrid forms of species management better suited to present-day conditions.

Examples abound of the new challenges

Modern conservation management increasingly integrates approaches developed in zoos with those from the wild to actively manage populations.

facing species conservation. Novel and emerging diseases threaten wildlife populations that will require new, active methods of veterinary management (3). The effects of climate change are driving us to consider moving populations of species outside their current geographic ranges to new areas where they may survive (4). In these circumstances, the view that species can be effectively conserved with minimal management simply by creating large areas of natural habitat no longer holds true.

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Species management in zoos has also faced substantial challenges. For decades, modern, accredited zoos have worked to maintain sustainable populations in their facilities. Yet recent work has shown that attempts to sustainably manage populations in zoos in isolation from the wild are succeeding for only a small number of species. In his sobering critique, Lacy (2) states that many of the most valued and often irreplaceable captive breeding programs will probably not meet the goals necessary to ensure that they remain as healthy, genetic representatives of the wild populations because of their small population sizes, unsuccessful breeding, inappropriate founding popu-

ing technologies, behavioral enrichment, and training and conditioning.

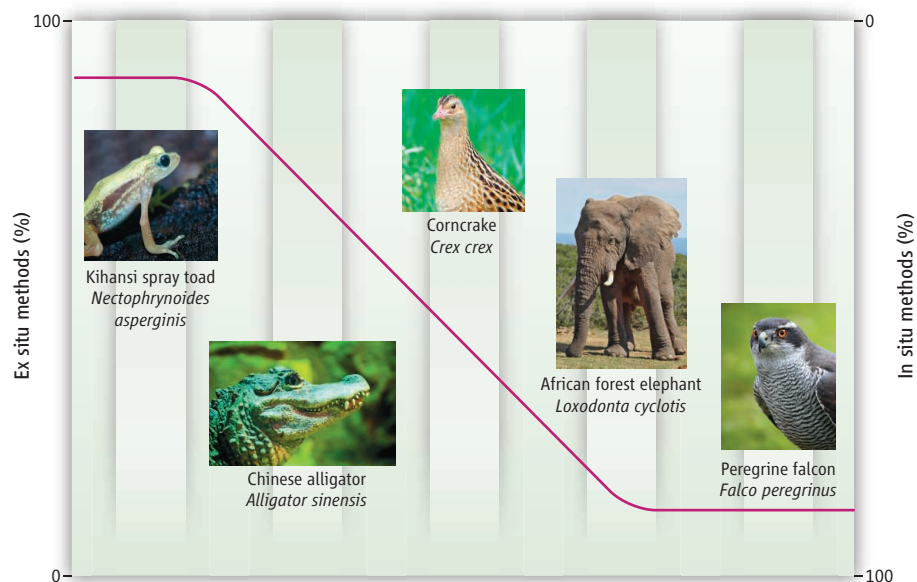
Practitioners of species conservation are increasing their use of approaches that integrate in situ and ex situ methods, effectively blurring the distinction between the two (see the figure). Tools and approaches that were developed or tested in zoos are invaluable with wild populations for relocations, in situ breeding, management, or soft releases (where there is supplemental care after the animal is released). Such hybrid approaches include, for example, supplementation of wild butterfly populations with captive-raised pupae (7); giving turtles a “head start” with a year or more of captive

stop managing species in order to maintain the richness and diversity we hold in such esteem. Scott *et al.* (15) have shown that conservation of 84% of the species listed under the U.S. Endangered Species Act will require continuing, species-specific management. These species have become “conservation reliant,” requiring a mix of conservation approaches (16). Many of the techniques required for such management will come from the zoo community with its experience in active, intensive management of individuals and small populations. Moreover, zoos will continue to play a leadership role in conserving severely threatened groups such as amphibians and turtles, some of which are under intense and immediate threat of extinction unless assurance colonies are created in captive settings (17).

The artificial divide between in situ and ex situ conservation has been a difficult one to bridge. The bridge will need to be built including new techniques that will include social science methods piloted in zoos. Most importantly, the development of novel methods needs to be led by decisions about which species are a priority for integrated conservation efforts.

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Blurred distinctions in conservation. Some species can be conserved through the use of largely ex situ methods, where animals are removed from their natural habitats, often in captive settings. Others require a mix of methods, and yet others require largely in situ methods, where species remain in their natural habitat.

lations, and poor cooperation in managed breeding programs. He and others (5, 6) have argued that captive populations should not be viewed as closed but should be managed as metapopulations linked to both wild and semi-wild populations.

The need to link zoo populations to wild populations through selective exchange of animals and/or their DNA demonstrates that zoos by themselves are not effective asarks for many targeted species (5, 6). Zoos are not merely contributors of animals from ex situ populations; they are also a source and testing ground for management techniques and approaches. They have been instrumental in the development and application of small-population management, health assessment and treatment, sedation and transport, contraception, reproductive studies, vaccine development, remote sens-

hatching and growth to avoid predation, then placing them in wild populations (8); provision of artificial nests for wild birds and removal of the resulting eggs to be raised in captivity for wild release, thereby inducing females to raise another clutch in the wild (9); integration of captive-bred animals into wild social groups before moving them to unoccupied parts of their range (10); transport of individual animals between populations to minimize inbreeding (11); capture, medical treatment, and release of individual wild animals (12); transport of resistant individuals from otherwise diseased populations to establish new disease-resistant populations (13); and collection of wild-animal gametes, which are combined in vitro and then implanted in captive animals so that the young can be released in the wild (14).

Humans will likely never be able to

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MATERIALS SCIENCE

LEGO-like DNA Structures

Kurt V. Gothelf

Parallels are often drawn between the construction of molecular structures and building toy models in LEGO bricks. This analogy is particularly suitable for the method reported by Ke *et al.* on page 1177 of this issue (1). The authors describe the construction of arbitrary and discrete three-dimensional (3D) DNA structures by self-assembly of single-stranded DNA “bricks.” The method opens a new route to complex self-assembled (3D) nanostructures that may serve as addressable templates for placing guest molecules with high precision, with possible applications in biophysics, medicine, and nanoelectronics.

electronic devices (3). The field has since advanced from assembling a few sequences to the assembly of large periodic lattices of repeated DNA tiles (4, 5). However, the formation of large discrete DNA nanostructures with unique tiles in all positions posed a major challenge. The conventional wisdom was that such structures were difficult to form owing to the requirement for exact stoichiometry of the DNA strands and the formation of noncompatible partial structures.

In 2006, Rothemund overcame this problem through the development of DNA “origami” (6). Using a long single-stranded DNA sequence as a scaffold and over 200

DNA “bricks” consisting of short sequences of single-stranded DNA self-assemble into a wide variety of shapes.

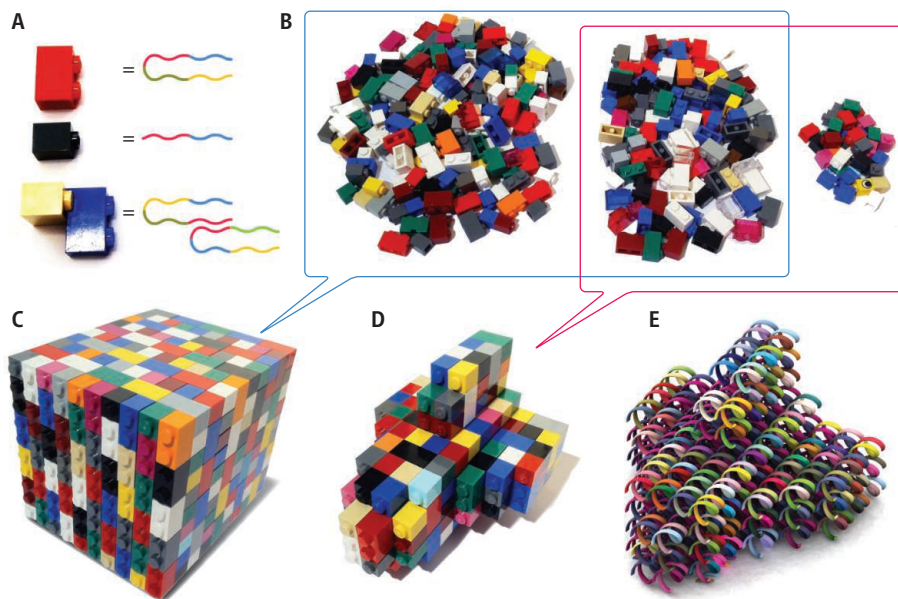
DNA origami is that the routing of the DNA scaffold in origami structures requires a new design and a new set of staple DNA strands for each new structure. Furthermore, the assembly of 3D origami structures is often slow and the yields low.

Yin and co-workers have challenged the conventional wisdom by using short single-stranded tiles (SSTs) to form large structures without a scaffold strand. Compared to conventional DNA tiles, the tiles are small, single-stranded, and have no ordered structure before they are incorporated in the superstructure (10). Recently, they used hundreds of SSTs to construct 2D structures with sizes comparable to those of 2D DNA origami (11).

In the latest work (1), the authors extend single-stranded tiles to single-stranded bricks and show that DNA brick assembly is a powerful method for constructing 3D structures. They show that arbitrary prescribed structures can be formed by selecting different subsets of strands from the same common pool of DNA sequences, making the design process more straightforward and enabling easy automation of the synthesis.

The basic building block in the structures is a 32-nucleotide single-stranded DNA, which contains four regions that can hybridize to four neighboring DNA strands (see the figure, panel A). To build 3D structures in a periodic way (panels B to E), the core design rule in this study is that the DNA bricks (resembling two-stud LEGO bricks) are connected with a 90° left-handed turn, resulting in layers of bricks that are shifted 90° relative to each other. Whereas LEGO structures are assembled by hand, brick by brick, the DNA structure forms by self-assembly: Each DNA brick is encoded with an individual sequence that determines its position and allows the structure to assemble by hybridization of complementary sequences. In a one-step annealing reaction in a magnesium-rich buffer, the cuboid structure (see the figure, panel C) formed over 72 hours.

The great advantage of the DNA brick method is that arbitrary structures can be sculpted from the same batch of DNA bricks by leaving out specific bricks and protecting the boundaries of the truncated structures with boundary and protector bricks. For example, the space shuttle-type of



How to make DNA brick structures analogous to LEGO® brick structures. (A) A DNA brick consists of four regions of 8 nucleotides each and corresponds to a two-stud LEGO brick. Half-DNA-bricks corresponding to one-stud LEGO bricks are used for edges (1). DNA bricks are connected by an 8-base pair hybrid, causing a 90° shift between two layers. (B) Ke *et al.* used one- and two-stud bricks (represented by the LEGO bricks in the blue frame) to assemble a 10 by 10 by 10 voxel cuboid (12) (C). With subsets of the bricks used for the cuboid, the authors also assembled many other shapes, such as a space shuttle-like structure, shown both as a LEGO (D) and DNA model (E). The extra bricks in the red-framed section in (B) are the boundary and protector bricks required for formation of the space shuttle structure.

DNA nanotechnology evolved from Seeman's pioneering ideas in the 1980s about using immobile DNA junctions to create periodic DNA lattices (2) and bio-

shorter synthetic strands as “staples” to fold the scaffold, he assembled arbitrary and discrete 2D DNA nanostructures with dimensions of typically ~100 by ~100 nm.

The DNA origami method overcame stoichiometry problems by allowing use of the short synthetic DNA strands in excess. Later, the method was extended to 3D DNA structures (7–9). However, one drawback of

CREDITS: (PANELS A TO D) KURT V. GOTHOLF; (PANEL E) FROM (1)

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structure (panels D and E) was created in this way.

Ke *et al.* demonstrate the generality and diversity of the method by creating 102 different structures out of subsets of the 10 by 10 by 10 voxel (12) cuboid. The structures have a wide variety of shapes, some with holes and closed cavities. There seem to be few limitations, except that a certain number of voxels must connect different parts of the structures to maintain the structural integrity.

It is puzzling that the structures form at all, instead of forming incompatible partial structures. The authors argue that seeding may be much slower than the growth of the structures, but it is not clear why this would be the case. Furthermore, the yields, at least for the larger structures, are relatively low.

Fortunately, the DNA brick and DNA origami methods are compatible; as Ke *et al.* discuss, it is likely that a combination of the two methods will pave the way for making even larger structures in higher yields.

Just as bricks of other shapes, other materials, and even small machines with matching sockets or studs can be integrated into LEGO structures, so can materials such as small molecules, nanoparticles, or proteins be integrated in DNA brick structures if these materials are conjugated to a DNA strand. Combined with the straightforward LEGO-like design principle of the DNA brick method and the possibility of integration with DNA origami, there is great potential for building advanced functional 3D nanostructures.

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ASTRONOMY

Embers of the Distant Past

Volker Bromm

Modern cosmology has come a long way in fulfilling its grand goal of reconstructing the entire history of the universe. The emergence of cosmic structure is governed by dark matter whose gravitational attraction led to the condensation of gas into galaxies, stars, and black holes. Whereas the evolution of the dark matter component is well understood (1, 2), cosmic star and black hole formation is still subject to large uncertainties. The combined energy production from all stars and black holes, releasing radiation when gas is falling close to their event horizon, however, is preserved in a pervasive cosmic radiation background—the extragalactic background light (EBL). This radiation comprises wavelengths from ultraviolet (UV) to far-infrared (IR) and provides us with an independent check on how many stars and active black holes have existed. On page 1190 of this issue, Ackermann *et al.* (3) present a new measurement of the EBL with the Fermi Space Telescope, based on the attenuation of distant sources of gamma-ray photons when they travel through the sea of lower-energy, background EBL photons.

Such a consistency check on the census of luminous sources also provides crucial constraints to close the gap in our cosmic

worldview, the first billion years after the Big Bang, when the first stars and galaxies formed (4). Our understanding of the first sources of light has to rely on theoretical studies, making predictions to be tested with the James Webb Space Telescope (JWST), to be launched around 2018. In the meantime, it is important to make progress by way of indirect constraints. Among them is the imprint left by the first stars on the EBL (5, 6). The basic process involves the ionizing photons emitted from massive primordial stars (see the figure), which are reprocessed into Lyman- α photons; they in turn travel through the expanding universe for billions of years, eventually being redshifted into the optical and infrared part of the spectrum.

Complementary to the Fermi results by Ackermann *et al.*, determining the strength of the EBL in the optical/UV, are measurements of the fluctuation power in the cosmic infrared background (CIB). Deep exposures with the Spitzer space telescope reveal hints of a highly clustered component that cannot be explained with any known sources, such as faint galaxies at intermediate redshifts (7). Light from the first stars and galaxies could account for the excess fluctuation signal, because the cosmological cold dark matter model naturally predicts that their formation sites are strongly clustered. A tantalizing new perspective has recently been provided by a possible correlation between fluctuations in the cosmic infrared and x-ray

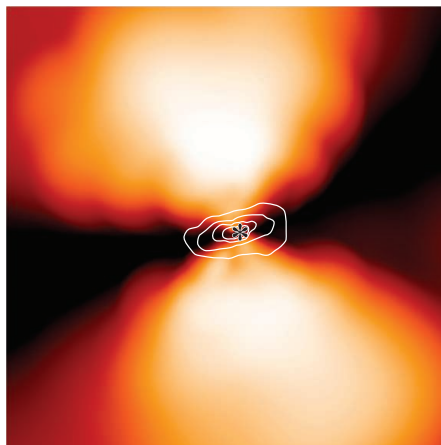
backgrounds (8). Such a cross-correlation could be naturally accommodated by models of first star formation, where the emergence of massive binary systems is predicted, provided that a fraction of them would evolve into x-ray binaries (9, 10). Such systems would contain a black hole remnant paired with a less-massive companion. The progenitor stars could contribute to the CIB, whereas the hard x-ray emission from their remnant would be deposited into the cosmic x-ray background. A correlated signal in the infrared and x-ray spectral region would naturally follow.

Both the Ackermann *et al.* Fermi and the CIB fluctuation results have as their prime goal to determine how many stars there were throughout cosmic time, including at high redshifts. This quantity is of crucial importance for a number of key questions in current cosmology. Among them is the problem of accounting for all the sources of ionizing radiation that, taken together, can reionize the universe at an age of about one billion years (11). There still seems to be a deficit, such that there must have existed an additional component of stars, or accreting black holes, in systems that are too faint, or too distant, to be detectable with current instrumentation. It is therefore crucial to constrain such “unseen” contributions. Theory suggest that at high redshifts, a mix of stellar populations was formed, including the elusive first generation of pure hydrogen/

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helium stars (Population III), and stars that formed out of material enriched with heavy elements, exhibiting more normal masses (Populations I and II). To solve the reionization puzzle, a key ingredient is to disentangle this mix of stellar populations. An intriguing clue has been provided by the discovery of a putative gas cloud that retained its primordial chemical make-up until two billion years after the Big Bang (12). This observation implies that unenriched islands may have persisted until after reionization was completed, thus allowing Population III stars to form even at more recent times.

Another promising diagnostic of the epoch of first light also depends on the rate of star formation at early times. This is to survey the sky for transient, explosive events that are sufficiently luminous to be detectable out to the very edge of the universe. Among them are gamma-ray bursts (GRBs) (13, 14). Such GRBs have been seen out to a redshift of 9.5, when the universe was just half a billion years old. However, the data quality was too poor to infer any of the vital details of the environment where the GRB took place, such as its heavy-element content and its degree of ionization. Similar information may be gleaned from observations of the first supernova explosions (15). Of particular prominence is the search for a superluminous class of explosions, called pair-instability supernovae. These require progenitor stars of very high masses, in excess of about 150 solar masses. A fraction of the first stars may have reached such masses, thus providing effective signposts of the early universe. For these surveys to succeed, the explosions and suitable



Radiation from the first stars. A supercomputer frame showing how one of the first stars is lighting up the early universe. A primordial star of 20 solar masses (marked by the star symbol) is located at the center of the frame, which has a total size of 40,000 times the average Earth-Sun distance. The high-energy radiation emitted by the star creates an hourglass-shaped region of hot, ionized gas (indicated by the bright colors). This ionized region is perpendicular to the circumstellar disk, outlined by the white contours. When the free electrons in the ionized region recombine with protons, Lyman- α photons are produced. These can escape into the surrounding intergalactic medium. After traveling through the expanding universe for about 13 billion years, in the process having their wavelength redshifted into the near-IR band of the spectrum, those photons contribute to the local EBL.

progenitor stars must have been sufficiently numerous. This is why the EBL constraints on the cosmic star formation rate provided by Ackermann *et al.* are crucial in mapping the cosmic landscape.

The next decade promises to open up a window into the very high redshift universe. New discoveries will arise from the confluence of next-generation observatories, such as the JWST and the planned extremely large ground-based telescopes, with ever more powerful supercomputer modeling. Already, we can catch a glimpse into the epoch of the first stars by scrutinizing the fossil imprint left behind in the various cosmic radiation backgrounds. Indeed, Fermi is in effect serving as such a pathfinder mission, setting the stage for a rapid period of discovery.

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EPIDEMIOLOGY

Outsmarting Outbreaks

Mark J. Walker and Scott A. Beatson

Everywhere, hospitals, clinics, medical centers, nursing homes, and other health care facilities face the possible spread of highly pathogenic or antibiotic-resistant bacteria within their premises. Measures are adopted to thwart this—infected patients are isolated, physicians and other staff members wear protective clothing and vigilantly wash their hands, exposed rooms are treated with dis-

infectant, and equipment and materials are sterilized or disposed of. Yet, there are about 100,000 deaths annually attributed to infections acquired at such facilities in the United States alone (1), and retrospective reports this year indicate that such measures are not sufficient. Incidents of the spread of deadly microbes both within hospitals and then, alarmingly, beyond those boundaries are an urgent reminder that strategies to stem outbreaks on a local scale must include effective procedures within the environment of patient care facilities. These procedures must keep pace with state-of-the-art tech-

Bringing advanced genomic sequencing technologies to health care facilities will help to prevent local outbreaks of dangerous pathogens.

niques that can track microbes in real time. Genomic sequencing can provide information that gives facilities a head start in implementing preventive measures. However, hospitals must be funded, staffed, and equipped to do this kind of investigation and analysis in real time.

The completion of high-quality reference genome sequences for the majority of important human pathogens has undoubtedly facilitated deeper understanding of disease mechanisms. High-throughput DNA sequencing technologies have been applied to study the emergence of epidemic strains

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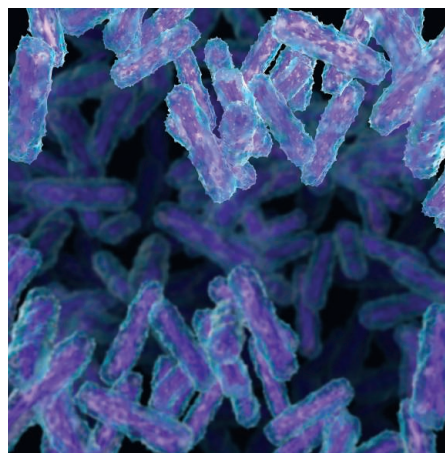
(such as *Escherichia coli* O104:H4 and *Streptococcus pyogenes*) (2, 3), the evolution of bacterial pathogens (4–7), microevolution within a single patient (8), and constituent microorganisms of the human microbiome (9). Genomic information has been applied to the development of new diagnostics and treatments, such as the identification of vaccine antigens for many dangerous pathogens including *Neisseria meningitidis* (10), which brings about meningococcal diseases and is a major cause of morbidity and mortality during childhood.

Now, the application of high-throughput genome sequencing to microbial infectious disease isolates is poised to robustly monitor infection transmission in the clinical setting and revolutionize current practice in clinical microbiology (11, 12). Indeed, recent reports show how genomic information can be used to track disease spread and control infection in hospitals (13–15). In 2011, an outbreak of carbapenem-resistant *Klebsiella pneumoniae* (see the figure) occurred in the U.S. National Institutes of Health (NIH) Clinical Center, with 11 patient deaths. Analysis of single-nucleotide polymorphisms (SNPs) shared by individual isolates enabled researchers to reconstruct the pathways of transmission during the outbreak. By identifying several SNPs that distinguished isolates taken from the “index” patient (patient of origin) at different time points, and integrating genomic and epidemiological data including interactions between people during the outbreak, the authors identified three transmission events from the index patient (13). Genomic data revealed transmissions that were not revealed by other means. In a real-time clinical situation, this information would enable further targeted testing of other patients or health care professionals to identify intermediate carriers. Alarming, one transmission event was linked to a contaminated ventilator used by two different patients—a clear demonstration of the capacity of genome sequencing–based epidemiology to influence hospital management practices.

Likewise, genome sequencing was used to investigate group A streptococcus (GAS; *S. pyogenes*) isolates associated with cases of puerperal sepsis in Australian hospitals in 2010 (14). *S. pyogenes* is estimated to cause about 700 million infections a year and more than 500,000 deaths (16). Whereas sequencing of the *emm* gene (encodes the major virulence determinant M protein) demonstrated that five of nine patients had acquired different GAS variants, it was not clear how the remaining four GAS isolates

were related to each other until genome sequencing was applied. Genome comparisons showed transmission of a strain between patients admitted to the same ward, but ruled out intra-institution and inter-institution transmission of the same strain to two other patients (14).

A larger study investigated an outbreak of methicillin-resistant *Staphylococcus aureus* (MRSA) in a neonatal intensive care unit in a hospital in Cambridge, UK. *S. aureus* bacteria are spread by skin-to-skin contact. MRSA is more difficult to treat because there are fewer effective antibiotics available for clinical use. The authors used a rapid bench-top sequencer, which poten-



Personalized microbial epidemiology. Recent hospital outbreaks of dangerous pathogens such as antibiotic-resistant *Klebsiella pneumoniae* indicate that advanced genomic sequencing technologies can inform policies at health care facilities to prevent their spread.

tially enables data delivery within a clinically relevant time scale (15). MRSA isolates from 20 patients were sequenced and the data formed two clusters, clearly discriminating between outbreak and non-outbreak MRSA, including some non-outbreak isolates with the same sequence type from other parts of the hospital. The report highlights the utility of genome sequencing data in place of multiple other genetic data that may take a relatively longer time to amass, such as antibiotic resistance and virulence gene profiles (these would normally be acquired through multiple polymerase chain reaction–based or phenotypic assays such as antibiotic susceptibility or antigen detection tests) (15).

These retrospective studies provide valuable lessons and point to a future in which direct sequencing of clinical samples allows same-day diagnosis, antibiotic resistance gene profiling, and virulence gene detection

for both single and mixed human infections. Technological impediments to the rollout of genomic methodologies in clinical laboratories include the time required to culture isolates before DNA preparation; the cost, time, and expertise needed to run sequence reactions using existing technologies; and the requirement for automated analysis pipelines to deal with genomic data produced on a routine basis and provide clinically meaningful reports (11, 12). Most hospitals are simply not equipped to generate and analyze such data. Culture of pathogens from clinical samples will remain indispensable in the short to medium term for many reasons: as a means of microbial identification; as a basis for phenotypic antibiotic resistance profiling; as a strain collection resource for retrospective and prospective research; and as a source of genomic DNA for parallel clinical and epidemiological studies. It offers low cost and quick turnaround, and can reliably determine phenotype even when the underlying genetic cause isn't known, such as in some cases of antibiotic resistance (11).

Ideally, an ultimate goal is to prevent the rise of so-called “superbugs.” But we can also become smarter about containment and local outbreak strategies. Through genomics, infectious agents can be unambiguously traced to individual patients, health care workers, and environmental niches; hence, focused infection control measures can now be adopted. The sequencers are readily available but we lack the established protocols and “clinic-ready” software needed to support wider adoption of this technology if we hope to limit outbreaks and improve patient care.

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RETROSPECTIVE

E. Donnall Thomas (1920–2012)

Frederick R. Appelbaum

With the death of E. Donnall (Don) Thomas on 20 October, the field of oncology lost an inspiring figure and the individual most responsible for developing human bone marrow transplantation. Don had what seemed to him a simple idea: Because normal marrow was easily destroyed by irradiation, shouldn't it be possible to destroy an abnormal marrow and replace it with marrow from a normal donor? Although simple in concept, Don's struggle to make marrow transplantation a clinical reality was long and difficult. Early attempts were unsuccessful, either because the transplanted marrow was rejected, or because the marrow rejected its new host. These failures led many to criticize Don's efforts as dangerous and futile. But his persistence paid off in the development of a therapy that has saved hundreds of thousands of lives.

Don became interested in hematopoiesis while training at Harvard Medical School (where he earned an M.D. in 1946). He witnessed Sydney Farber's first use of antifolates to induce remission in acute leukemia and Allan Erslev's search for erythropoietin. He also learned of Leon Jacobson's studies showing that shielding the spleen or bone marrow protected mice from the lethal effects of irradiation. When studies showed that irradiated mice infused with donor marrow could recover, Don was convinced of the potential of human marrow transplantation.

In 1955, Don moved to the Imogene Bassett Hospital in Cooperstown, New York, to work with Joseph Ferrebee. They published the first experience with human allogeneic marrow transplantation. Six patients were treated with radiation and chemotherapy followed by infusion of normal donor marrow. These were only transient grafts and none lived beyond 100 days. Attempts at allogeneic marrow transplantation by others, including some in victims of a nuclear-reactor accident, also failed. Because histocompatibility was not understood and no efforts were made to match donor and recipient, these results are not surprising.

Yet Don remained convinced of the potential of marrow grafting and turned to a canine model. He found that after total body irradiation

and infusion of littermate marrow, most dogs developed the problems seen in humans, including graft rejection or graft-versus-host disease. But some dogs became long-term survivors with marrow completely of donor origin. Don reasoned that the successful donor-recipient pairs were somehow genetically matched and that discovering methods for donor selection was critical. In 1963, he moved to Seattle, where he and his colleagues developed rudimentary canine histocompatibility testing. They also determined appropriate doses of radiation to ensure engraftment



and developed methotrexate postgrafting to prevent graft-versus-host disease. By the mid-1960s, they could reliably transplant canine littermates. Meanwhile, methods for human leukocyte antigen typing became available and, with these advances, Don returned to the challenge of human marrow grafting.

He assembled a team of physicians, nurses, and technicians and in 1969 began clinical trials of allogeneic transplantation from matched siblings for patients with advanced leukemia. They went to extraordinary lengths to support patients, housing them in sterile laminar airflow rooms, asking staff members to donate platelets, and working with Robert Hickman to develop a catheter for intravenous alimentation. Most of the initial patients died of progressive leukemia or complications of transplantation, but a few survived. In 1975, now at the newly formed Fred Hutchinson Cancer Research Center,

A physician's unrelenting commitment made bone marrow transplantation an effective cancer therapy.

Don published results showing a plateau in the survival curve, demonstrating that a minority of patients with otherwise incurable leukemia had been cured. The group started transplanting patients earlier in their disease, while in remission, and soon was reporting cure rates in excess of 60%. This success led the Seattle team to explore transplantation for other diseases of hematopoiesis, including sickle cell anemia, thalassemia, myelodysplasia, multiple myeloma, and lymphoma.

Transplantation was initially limited to the approximately 25% of patients with a matched family member. In the late 1970s, the Seattle group performed the first successful marrow transplant from a matched unrelated donor. This success encouraged the creation of donor registries in the United States, Europe, and Asia. Remarkably, more than 20 million potential donors are now typed and listed in these registries. With the broadened indications for transplantation and the increased availability of donors, the use of marrow transplantation has steadily grown. This year, about 65,000 transplants will be performed worldwide, and the cumulative number of transplants performed since Don began his work will surpass 1,000,000.

Don was born on 15 March 1920 in Texas. He liked to mention that between him and his father (a physician), they spanned the period from horse-and-buggy house calls to modern high-tech medicine. He studied chemistry at the University of Texas where he received his B.A. and where he met his wife, Dorothy (Dottie). Besides raising three children together, Dottie was Don's partner in every aspect of his professional life, from working in the laboratory to editing manuscripts and administering his research program. If Don was the father of marrow transplantation, then Dottie is surely the mother. For his work, Don deservedly received a multitude of awards, including the 1990 Nobel Prize in Physiology or Medicine, which he shared with Joseph Murray. He was equally admired for his pioneering scientific discoveries and the way in which he achieved his success. He was hard-working, uncompromising, and a demanding critic. But he was also humble, witty, generous with ideas, and quick to deflect praise to his co-workers. Most of all, he was dedicated to his patients and the development of a therapy to give them a better chance at life.

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CREDIT: SUSIE FITZHUGH

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IBI* SERIES WINNER

Solving Complex Problems

K. V. Hodges

Before he became America's first de facto science adviser and before he helped lay the foundation for the National Science Foundation, Vannevar Bush was a professor of Electrical Engineering and, eventually, dean of Engineering and vice president at the Massachusetts Institute of Technology (MIT). In those capacities, he came in contact with some of the nation's best and brightest minds in their formative years. But after two decades in such a rarified academic environment, Bush had become disenchanted by the increasing specialization of undergraduate curricula in science and engineering in America (1). He felt that education in these fields placed too much emphasis on information transfer from teacher to student and too little on deep understanding and intellectual synthesis by the student. Bush was among the first to anticipate that massive amounts of information would someday be universally and readily available to all, such that our ability to communicate knowledge through classes would become far less important than our ability to inspire students to do something creative, and valuable, with it.

Invented at MIT some 60 years later and first offered in 2000, "Solving Complex Problems" is a class designed to do just that (2). A freshman-year elective for students with a wide range of backgrounds and prospective majors, it typically attracts between 5 and 10% of the MIT freshman class who develop through it an enthusiasm for tackling difficult, multifaceted problems. Students are presented in the first class with a challenge that can be stated simply, but that is deceptively complex and has no straightforward answer. Over the course of the semester, it is their job collectively to "imagineer" a proposed solution, to articulate their solution, and to explain how they arrived at it.

For example, the challenge presented to the first class in 2000 was to design a mission of exploration to Mars to search for signs of past or present life. Some students, who saw themselves as prospective aeronautics or astronautics majors, immediately inter-

preted this as a simple invitation to solve the ideal rocket equation for the appropriate thrust necessary to transfer a research payload to Mars and back. But it soon became clear that the simplicity of the problem statement masked a spectrum of challenges that would require the development and analysis of complicated decision matrices. Some of the implied questions were fundamental. How should we define "life" for the purpose of this mission? If one uses the life we know on Earth to establish what to look for, how can we be sure that a search for traditional biosignatures is sufficient to conclude



Establishing the learning environment. Upper-class mentors prepare for the week's student team meetings at MIT.

that life does not exist on Mars? The phrase "past or present" life adds more complexity to the task. What do we regard as reliable evidence for fossil life? Other questions were more operational. Should the mission be manned or unmanned? How should the spacecraft be designed? What analytical instruments would be best for the required measurements? Still others were eminently practical, including the two most practical of all: How much will all this cost, and who will pay for it?

Other missions presented challenges of different kinds. In 2001, a sufficiently large number of students enrolled that it became practical to split the core mission—to design a permanently manned, environmentally sensitive undersea research laboratory and to develop research plans for the first 6 months of the operation—into two. The Atlantis I class section was charged with designing a research station that would be located in the Belize barrier reef complex,

Solving Complex Problems, an IBI prize-winning module, challenges students to use their knowledge creatively in order to design solutions.

whereas the Atlantis II station would be on the sea floor at the Edmond hydrothermal vent field in the Indian Ocean at a depth of more than 3000 m. The Atlantis I section soon found that environmental sensitivity would be of paramount importance for success, and the students had to deal with issues as varied as how to deal with waste, how to maintain a stable ocean temperature around the station, and even whether or not the station should be permanently anchored or neutrally buoyant to protect the reef substrate. In contrast, the Atlantis II section had to design for an environment with no natural light and where hull pressures on the station would be extreme. (The students settled on a torus-shaped station with an internal diameter of 6 m and wall thicknesses of 36 cm.) An added value to the two-section structure was that students from both sections met over the course of the semester to compare differences and similarities in their designs and the challenges they faced.

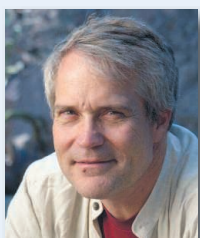
Regardless of topic, the students in a section of Solving Complex Problems all work together in the first few class sessions to predict what challenges will arise and to parse the overall problem into a series of 5 to 10 themes. For example, themes might include the environmental context of the problem, engineering challenges, public relations, budget development, and fund raising. Each student is then assigned, at random, not based on preference, to a team responsible for developing a knowledge base and making preliminary recommendations for their part of the overall solution. Perhaps surprisingly, we have found the approach of randomizing teams very effective because all teams ultimately have to work together on the final design concept; a student particularly interested in one theme—but not assigned to the team associated with it—is encouraged to act as a sounding board (and sometimes friendly critic) for the team. One member from each team is elected to be part of a coordination team to ensure good inter-team communications.

In the MIT implementation of Solving Complex Problems, these teams benefit from consultations with upper-class mentors, many of whom are themselves veterans of the class (see the first figure), and alumni mentors with special knowledge of one or

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*IBI, Science Prize for Inquiry-Based Instruction; www.sciencemag.org/site/feature/data/prizes/inquiry/.

About the author



Kip Hodges is a Foundation Professor at Arizona State University and Founding Director of ASU's School of Earth and Space Exploration. Before 2006, at MIT he was a Professor of Geology in the Department of Earth, Atmospheric, and Planetary Sciences; a MacVicar Faculty Fellow; and a Codirector of both the Earth System Initiative and Terrascope, a freshman learning community in which Solving Complex Problems plays a foundational role. He earned a B.S. in geology at the University of North Carolina at Chapel Hill and his Ph.D. at MIT. His research foci include continental tectonics, noble gas geochronology, and planetary field science (with a special emphasis on developing advanced protocols for scientific exploration of other worlds).

Tom Lovejoy, biodiversity chair at the Heinz Center for Science, Economics, and the Environment; (ii) representatives from Raytheon who were working on the Brazilian government's System for the Preservation of the Amazon; and (iii) Larry Linden, founder of the Linden Trust for Conservation. All panelists serve on a volunteer basis. Many of the MIT student Web sites and video archives of final presentations are available on the subject Web site (web.mit.edu/12.000).

The instructor's role in this class is primarily to create an environment conducive to self-directed learning (see the second figure). There

more aspects of the overall problem. Typically, about half of the upper-class mentors receive a small stipend for their participation, whereas the rest earn academic credit. Mentors generally spend 3 to 5 hours per week working in their roles. Alumni all serve on a volunteer basis, and we have found them to be very generous with their time, sometimes volunteering over a number of years to spend an average of an hour or two each month interacting with the students.

The upper-class and alumni mentors are instructed to serve as sounding boards and information resources but are asked not to be directly involved in the design process. As the project evolves, the teams naturally coordinate their efforts better to achieve an optimal overall design. At the end of the semester, the design is documented as a content-rich Web site, and the students give a public presentation of the design to one or more experts who deal with such problems as professionals. As an example, the expert panelists for the 2002 class, which had as its focus the design of technological strategies to monitor the Amazon rainforest environment and to devise strategies to ensure its preservation, included (i)

are no lectures, although the students are exposed in a casual way to a series of case studies that are germane to their problem. For example, in the Mars problem from 2000, lessons learned from the Apollo program featured prominently. The students are instead encouraged to learn independently using a variety of resources, including the Web (with extensive coaching on how to recognize reliable and unreliable content); libraries; and self-motivated conversations

with faculty, graduate students, older undergraduates, and alumni. In the early years of offering this subject, we passed on to the students a list of people who had been recruited by the instructional staff and had volunteered to participate in such discussions. However, we soon found that such recruiting efforts were unnecessary; many at all

levels of the academic community are open to such informal interactions when they are precipitated by students asking questions that begin with a phrase like: "What is your take on ...?" These casual conversations are especially valuable because they impart an appreciation for practical integration of acquired knowledge. For example, freshmen in the "Mars exploration" year of

Solving Complex Problems benefited from a brainstorming conversation with alumnus Joe Gavin, who managed the engineering program at Grumman Aircraft that designed and built the Apollo lunar landing module, about the challenges posed by descent to and ascent from other planetary surfaces.

Along the way to arriving at their optimal design, the students learn valuable lessons regarding critical, transdisciplinary thinking, the challenges and rewards of working in teams both large and small, the importance of organizing and synthesizing data from many sources, and the need to justify assumptions and decisions. Early in the development of the class, we learned that a grading scheme was necessary that recognized individual accomplishment but rewarded collaborative problem solving. We allow students to critique their own work, the work of others on their thematic teams, and the class as a whole. But the final grade for the semester depends disproportionately on the quality and sophistication of the overall design as judged by the teaching staff with input from the expert panelists.

The Solving Complex Problems learning environment has proven to be extremely adaptable. Since leaving MIT in 2006 to take up my current position at Arizona State University (ASU), I have used the same approach with more advanced students for a required sophomore-junior-level subject in the B.S. degree in the School of Earth and Space Exploration. In the Spring of 2013, I will use it in teaching the senior capstone subject for our unique B.A. degree in Earth and Environmental Studies, which is designed to emphasize science literacy for liberal arts students who do not anticipate careers in science and engineering. Solving Complex Problems and its curricular descendants provide students with an opportunity to integrate many modes of inquiry, from science and engineering, to public policy, education, economics, and even media affairs. I think Vannevar Bush would approve.

References and Notes

1. G. P. Zachary, *Endless Frontier: Vannevar Bush, Engineer of the American Century* (Free Press, New York, 1997).
2. A video introduction to Solving Complex Problems, as well as more information and a general syllabus, are available through MIT's OpenCourseWare site: ocw.mit.edu/courses/earth-atmospheric-and-planetary-sciences/12-000-solving-complex-problems-fall-2003

Supplementary Materials

www.sciencemag.org/cgi/content/full/338/6111/1164/DC1

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SCIENCE POLICY

Looming Budget Cuts Threaten U.S. Research Advances

For decades, U.S. researchers have been working to solve the elemental secrets of human health and disease: the function of the immune system, the triggers for cancer, the remarkable plasticity of pluripotent stem cells. Today, says University of Pennsylvania Senior Vice Provost for Research Steven J. Fluharty, the life sciences have come to a transformative moment—only to find historic progress threatened by deep federal budget cuts that could be just weeks away.

At a Capitol Hill briefing organized by AAAS, Fluharty joined other science leaders urging Congress and the White House to avert the “sequestration” that could slash the U.S. investment in research and development by 8.4%—some \$58 billion—by 2018. Cuts of that magnitude, they said, would jeopardize work in areas such as genetic medicine, advanced manufacturing, and batteries that could allow a 10-fold increase in the range of electric cars.

“We’re talking about dramatically reducing the rate of discovery and innovation in this country, which has traditionally been the lifeblood of our economy,” said Fluharty.

The 14 November briefing reflects broad outreach by the U.S. science and engineering sector that has intensified as each day gets closer to the so-called fiscal cliff. As elected officials search for a debt-reduction compromise by year’s end, both parties have signaled support for research investment.

AAAS has been prominent in the effort, providing detailed budget analysis, building alliances with other S&T organizations, and working with lawmakers. The AAAS Office of Government Relations has established a Web site to provide background on the potential budget cuts (www.aaas.org/go/sequester). And AAAS Chief Executive Officer Alan I. Leshner has detailed the importance of federal R&D in publications ranging from the *Washington Post* and the *Sacramento Bee* to Germany’s weekly *Die Zeit*.

“AAAS is a leading voice for the scientific enterprise,” Leshner said, “and we have a responsibility to ensure that lawmakers and the public understand the severe consequences if these cuts take effect.”

The sequestration deadline is the result of a 2011 compromise among lawmakers that averted a budget crisis by allowing an increase in the federal government’s debt ceiling. Under the deal, if lawmakers can’t reduce the deficit on their own, automatic sequestration cuts kick in.

Matt Hourihan, director of the AAAS R&D Budget and Policy Program, described for the bipartisan, standing-room-only Capitol Hill audience the potential impact on top federal science agencies. At the Department of Defense, R&D would fall 9.1%, or \$33.5 billion, over 5 years. The Department of Energy would lose 8.2%, or nearly \$4.6 billion. Both the Department of Agriculture and NASA would lose 7.6%, with NASA’s R&D funding falling to its lowest level since 1988.



A plan approved in the U.S. House of Representatives would exempt defense from sequestration, but that would mean cuts of 17.5% for nondefense R&D. While that’s unlikely to advance, profound concerns have galvanized science and engineering associations, universities, and others in the U.S. research community.

One key focus: the projected cut of 7.6%, or \$11.3 billion, to R&D at the National Institutes of Health (NIH). Research!America, the medical and health research advocacy alliance, reported 77% of respondents to its recent national public opinion poll favored federal funding for research to improve the health care system. Nearly 70% favor increased federal support for scientific research that advances knowledge and drives innovation.

The Federation of American Societies for Experimental Biology warned that the cuts would be “devastating.” NIH would fund 2300 fewer medical research grants in 2013, the federation said, forcing lab closures and tens of thousands of layoffs. Earlier this month, its call for action generated more than 8000 e-mails to Congress.

Whether this combined effort is as large as past campaigns is uncertain. “There are so many more players today than there were back in the 90s, and we’ve learned a lot of lessons,” said Joanne Carney, director of the AAAS Office of Government Relations. “We’ve matured, and we have a more sophisticated view of how best to advocate for science.”

But the message this fall is inherently pragmatic: Reduced funding means reduced discovery and reduced benefits for society. And that could undermine a new generation of scientists and engineers just as they are coming into the professions. The nation can’t afford such losses, experts said at the Capitol Hill briefing.

The world is growing more competitive, and other countries are investing heavily in R&D, said Orlando Auciello, formerly of Argonne National Laboratory and now Endowed Chair Professor at the University of Texas at Dallas. “So you have to tell your representatives in Congress: ‘This is not red or blue... If we don’t work for the United States of America, we’re going to be in trouble.’”

Uzbek, U.S. Scientists Plan to Expand Partnerships

Top U.S. and Uzbek scientists explored potential research collaborations in human health, agriculture, and environmental sciences at a 3-day conference convened by AAAS in Tashkent.

Uzbek and U.S. scientists have worked together previously on cotton and clean water research, and participants from both countries were enthusiastic about expanding collaborations in genomics, proteomics, and climate change.

Microbiology and solar energy research are also emerging as promising fields for cooperative science, said Norman P. Neureiter, senior adviser to the AAAS Center for Science Diplomacy and acting director of the AAAS Center for Science, Technology and Security Policy. "Uzbekistan has a collection of opportunities for considerable cooperative work," he said.

These opportunities have grown steadily since a 2010 Science & Technology Cooperative Agreement signed by the U.S. and Uzbekistan, as well as a 2011 visit to the country by U.S. State Department science envoy and former AAAS Board member Alice P. Gast.

More than 70 researchers and dignitaries, including Shavkat Salikhov, president of the Academy of Sciences of the Republic of Uzbekistan, and U.S. Ambassador George Krol, attended the 27 to 29 September meeting. Former AAAS President Gilbert S. Omenn and current AAAS Board member Inder Verma also spoke at the conference; it was organized by Gwenaële Coat, a senior program associate at the AAAS Center, and Uzbek Academy researcher Ibromkhim Abdurakhmonov.

The role model for many gathered in Tashkent is a decade-long collaboration in cotton genetics between the U.S. Department of Agriculture (USDA), Texas A&M University, and the Uzbek Academy of Sciences. Abdurakhmonov and Texas A&M's Alan Pepper described how the program has produced high-quality studies and supported a remarkable cotton-breeding program in a country at the same chilly latitude as Chicago.

Joy Ward, a University of Kansas professor, is pursuing collaborations with senior Uzbek scientists to focus on important plant genes that may respond to environmental change. She and many others also spoke about expanding international opportunities for the next generation of Uzbek researchers. "We need to bring more early-career

scientists to conferences like this one, as they are the hope for continued collaborations between our nations."

Many of the researchers noted the need for funding to move these partnerships beyond the talking stage, said Jacqueline Fletcher, an Oklahoma State University scientist who pointed to the USDA program as an inspiring example. "Uzbek and U.S. scientists share many common goals and productive interactions are often easy to envision, but consistent

financial resources are needed to engage and continue that engagement over time," she said.

"I think, currently, we—both the U.S. and Uzbek sides—must concentrate on finding the funding resources for the collaborations intended," Abdurakhmonov agreed. "This requires good ideas and continual discussion."

The researchers plan to continue their discussions and define specific cooperative projects at a meeting in the United States in 2013.

—Becky Ham

COMMUNICATION

AAAS Kavli Science Journalism Award Winners Named

Stories about microbial hitchhikers, the largest dam-removal project in North America, and issues raised by the new era of personal genomics are among the winners of the 2012 AAAS Kavli Science Journalism Awards.

Large Newspaper—(Circulation of 100,000 or more): Carl Zimmer, freelance writer, for stories published in the *New York Times*: "Evolution Right Under Our Noses" (26 July 2011); "A Sharp Rise in Retractions Prompts a Call for Reform" (17 April 2012); and "Tending the Body's Microbial Garden" (19 June 2012). The judges praised Zimmer's entry as an example of sustained excellence in reporting on a range of science topics.

Small Newspaper—(Circulation less than 100,000): The judges declined to give an award in the small newspaper category this year.

Magazine: Michelle Nijhuis, freelance writer, for "Crisis in the Caves," published July/August 2011 in *Smithsonian* magazine. Nijhuis went underground to observe both bats and biologists as she reported on white-nose syndrome, which has killed more than a million cave-dwelling bats in the northeastern United States.

Television—(Spot News/Feature Reporting, 20 minutes or less): Sheraz Sadiq, KQED QUEST (San Francisco), for "Hetch Hetchy Aqueduct: Big Fixes for Big Quakes," 9 November 2011. With historical footage and on-the-scene reporting, Sadiq explained the engineering steps being undertaken to protect the San Francisco Bay Area's water supply.

Television—(In-Depth Reporting, more than 20 minutes): Sarah Holt and Laurie Donnelly WGBH/NOVA, for "Cracking Your Genetic Code," 28 March 2012. The journalists' entry told about the emerging field of personalized medicine—where success and failure often

intermix—through the eyes of a cancer patient, a cystic fibrosis sufferer, and others.

Radio: Bari Scott, Alex Chadwick, Mary Beth Kirchner, Robert Rand, and Robin Wise, Sound-Vision Productions for American Public Media, for "Particles: Nuclear Power After Fukushima," 11 March 2012. The program revisited the Fukushima disaster to show "energy issues through the lens of personal experience."

Online: Lynda V. Mapes, Steve Ringman, and Genevieve Alvarez, *The Seattle Times*, for "Elwha: The Grand Experiment," 17 September 2011. Their series covered the \$325 million project to remove two dams that have blocked salmon runs for more than a century on the Elwha River and to restore 800 acres of former reservoirs in Washington State's Olympic Peninsula.

Children's Science News: Kirsten Weir, freelance writer, for "Uninvited Guests," published in *Current Health Kids*, April/May 2012. Weir mixed compelling statistics and humor in her lively tour of the trillions of microbial stowaways on the human body.

The awards, administered by AAAS since their inception in 1945, go to professional journalists for distinguished reporting for a general audience. The Kavli Foundation provided a generous endowment in 2009 that ensures the future of the awards program.

Independent panels of science journalists pick the winners, who will receive \$3000 and a plaque at the 2013 AAAS Annual Meeting in Boston in February. Learn more about the winning entries at www.aaas.org/sja2012.

—Earl Lane

ASSOCIATION AFFAIRS

AAAS Members Elected as Fellows

In October 2012, the AAAS Council elected 701 members as Fellows of AAAS. These individuals will be recognized for their contributions to science and technology at the Fellows Forum to be held on 16 February 2013 during the AAAS Annual Meeting in Boston, Massachusetts. The new Fellows will receive a certificate and a blue and gold rosette as a symbol of their distinguished accomplishments. Presented by section affiliation, they are:

Section on Agriculture, Food, and Renewable Resources

James R. Alfano, Univ. of Nebraska-Lincoln
 Nilsa A. Bosque-Pérez, Univ. of Idaho
 Richard M. Bostock, Univ. of California, Davis
 Edward S. Buckler, Cornell Univ.
 Yves Carrière, Univ. of Arizona
 Mary Erin Delany, Univ. of California, Davis
 Kellye A. Eversole, Eversole Associates
 Mark Lawrence Failla, Ohio State Univ.
 John James Finer, Ohio State Univ.
 Avtar Krishan Handa, Purdue Univ.
 Maria J. Harrison, Cornell Univ.
 James W. Jones, Univ. of Florida
 Karen E. Koch, Univ. of Florida
 Weiping Liu, Zhejiang Univ., China
 Cathie Martin, John Innes Centre
 William B. McGill, Univ. of Northern British Columbia, Canada
 Ravi Naidu, Univ. of South Australia
 Kerry O'Donnell, USDA-ARS
 Melvin J. Oliver, USDA-ARS
 N. LeRoy Poff, Colorado State Univ.
 Sanjaya Rajaram, Resource Seed Mexicana
 Matthew Brian Thomas, Pennsylvania State Univ.
 Michael Karl Udvardi, Samuel Roberts Noble Foundation
 Jonathan D. Walton, Michigan State Univ.
 Guoyao Wu, Texas A&M Univ.
 Kun Yan Zhu, Kansas State Univ.

Section on Anthropology

Arlen Frank Chase, Univ. of Central Florida
 Mark V. Flinn, Univ. of Missouri-Columbia
 Mary Anne Katzenberg, Univ. of Calgary, Canada
 Joanna E. Lambert, Univ. of Texas at San Antonio
 Patricia Lambert, Utah State Univ.
 Lisa J. Lucero, Univ. of Illinois at Urbana-Champaign
 Lorena Madrigal, Univ. of South Florida
 Herbert D.G. Maschner, Idaho State Univ.
 Elizabeth Jean Reitz, Univ. of Georgia, Athens
 Katerina Semendeferi, Univ. of California, San Diego
 Jan F. Simek, Univ. of Tennessee, Knoxville
 Peter Stuart Ungar, Univ. of Arkansas

Section on Astronomy

Lynn R. Cominsky, Sonoma State Univ.
 Eli Dwek, NASA Goddard Space Flight Center
 Bruce G. Elmegreen, IBM T.J. Watson Research Center
 Neal J. Evans II, Univ. of Texas at Austin
 Neil Gehrels, NASA
 Sun Kwok, Univ. of Hong Kong
 Angela V. Olinto, Univ. of Chicago
 Richard William Pogge, Ohio State Univ.
 Nathan A. Schwadron, Univ. of New Hampshire
 Keivan Guadalupe Stassun, Vanderbilt Univ.
 Michiel van der Klis, Astronomical Institute Anton Pannekoek, Netherlands
 G. Mark Voit, Michigan State Univ.
 Arthur M. Wolfe, Univ. of California, San Diego

Section on Atmospheric and Hydrospheric Science

Thomas Stephen Bianchi, Texas A&M Univ.
 Anny Cazenave, Centre National d'Etudes Spatiales (CNES), France
 Harindra Joseph Shermal Fernando, Univ. of Notre Dame
 Susan Joy Hassol, Climate Communication
 Brian John Hoskins, Univ. of Reading/Imperial College London, UK
 Robert A. Houze Jr., Univ. of Washington
 Andrew John Weaver, Univ. of Victoria, Canada

Section on Biological Sciences

Soman Ninan Abraham, Duke Univ. Medical Center
 Anurag Agrawal, Cornell Univ.
 Paul G. Ahlquist, Univ. of Wisconsin-Madison
 Susan C. Alberts, Duke Univ.
 Stephen Alexander, Univ. of Missouri-Columbia
 Edith Bach Allen, Univ. of California, Riverside
 Christopher I. Amos, MD Anderson Cancer Center
 Gynheung An, Kyung Hee Univ., South Korea
 Norman Arnheim, Univ. of Southern California
 Alexander V. Badyaev, Univ. of Arizona
 Diane L. Barber, Univ. of California, San Francisco
 James C.A. Bardwell, Univ. of Michigan
 Susan Schloemer Bell, Univ. of South Florida
 Shelley L. Berger, Univ. of Pennsylvania
 Albert H. Beth, Vanderbilt Univ. School of Medicine
 James D. Bever, Indiana Univ.
 John M. Blair, Kansas State Univ.
 Bruce Blumberg, Univ. of California, Irvine
 Susan Bonner-Weir, Joslin Diabetes Center
 Bruce A. Bowerman, Univ. of Oregon
 Susan H. Brawley, Univ. of Maine
 Charles Brenner, Univ. of Iowa
 Michael R. Brent, Washington Univ. in St. Louis
 Edmund D. Brodie III, Univ. of Virginia
 Yves V. Brun, Indiana Univ.
 Thomas P. Brutnell, Donald Danforth Plant Science Center
 Breck Edward Byers, Univ. of Washington
 Rafael Daniel Camerini-Otero, National Institutes of Health
 Jane M. Carlton, New York Univ.
 Nicholas C. Carpita, Purdue Univ.
 Daniel D. Carson, Rice Univ.
 Charles Williams Carter Jr., Univ. of North Carolina at Chapel Hill
 Patrick J. Casey, Duke Univ. Medical Center
 Susan E. Celniker, Lawrence Berkeley National Laboratory
 Roger Chalkley, Vanderbilt Univ. Medical Center
 Jianzhu Chen, Massachusetts Institute of Technology
 Zhijian 'James' Chen, Univ. of Texas Southwestern Medical Center
 Chi-Hing Christina Cheng, Univ. of Illinois at Urbana-Champaign
 Xiaodong Cheng, Emory Univ. School of Medicine
 Sallie Watson Chisholm, Massachusetts Institute of Technology

Ken W.Y. Cho, Univ. of California, Irvine
 Vitaly Citovsky, Stony Brook Univ.
 Nancy Hall Colburn, National Cancer Institute/NIH
 Luca Comai, Univ. of California, Davis
 Roger D. Cone, Vanderbilt Univ.
 Lynn Cooley, Yale Univ. School of Medicine
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Ice-Sheet Response to Oceanic Forcing

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The ice sheets of Greenland and Antarctica are losing ice at accelerating rates, much of which is a response to oceanic forcing, especially of the floating ice shelves. Recent observations establish a clear correspondence between the increased delivery of oceanic heat to the ice-sheet margin and increased ice loss. In Antarctica, most of these processes are reasonably well understood but have not been rigorously quantified. In Greenland, an understanding of the processes by which warmer ocean temperatures drive the observed retreat remains elusive. Experiments designed to identify the relevant processes are confounded by the logistical difficulties of instrumenting ice-choked fjords with actively calving glaciers. For both ice sheets, multiple challenges remain before the fully coupled ice-ocean-atmosphere models needed for rigorous sea-level projection are available.

There is long-standing concern about Greenland and Antarctica's contributions to rising sea level. Earlier projections indicated ice-sheet losses in Greenland would be offset largely by gains in Antarctica, where a warmer atmosphere is expected to increase snowfall more than surface melt (1). Such projections are at odds with recent observations, which indicate that both ice sheets are losing mass at accelerating rates. Increased ice discharge to the ocean (i.e., greater iceberg calving) has produced much of this increased loss as numerous glaciers and ice streams have sped up rapidly (months to years) over the past two decades (2, 3). Largely unanticipated as recently as a decade ago, many of these changes were observed just as the Intergovernmental Panel on Climate Change (IPCC) was preparing its Fourth Assessment, prompting the panel to conclude that they could place no upper bound on the ice dynamics-driven contribution to sea level (4).

The recent changes in ice flow have highlighted the important role that ice-ocean interaction plays in ice-sheet stability, which has sometimes been overlooked. The ocean's tremendous heat capacity means that shifting currents or warming water can substantially alter the rate of melting at the ice-ocean interface (5). This interaction is particularly important in Antarctica, where little melting occurs at the ice-air interface. By contrast, considerable (tens of cm to tens of meters per year) melting occurs beneath the continent's fringing ice shelves, which are floating extensions of the

grounded ice sheet (6). Removing ice from a land-terminating margin requires that heat be supplied to melt or sublimate ice in situ, limiting the rate of loss. Ocean currents, however, can rapidly carry away excess ice to melt elsewhere after it calves from an ice-ocean terminus in response to forcing at the boundary (e.g., oceanic or atmospheric heating), enabling rapid retreat. Lastly, the ice-sheet grounding line (Fig. 1), where the grounded ice sheet transitions to a floating ice shelf, often lies at a point of tenuous stability, such that small initial perturbations can trigger large-scale retreat (e.g., marine ice-sheet instability) (7).

Oceanic Setting

Along an ice-sheet periphery, the ocean surface waters tend to be relatively fresh and cold (Fig. 2, C and D), typically at or near the surface freezing point. The properties of such waters typically are of polar origin and have only modest impact on melting beneath ice shelves. Below these surface waters, at depths typically ranging from 100 to 1000 m, there often resides a relatively warm and salty layer of water originating from the subtropical or subpolar regions (Fig. 2, C and D). These water masses are referred to as Irminger Water (IW) and Atlantic Water (AW) south and north of Greenland, respectively, and as Circumpolar Deep Water (CDW) surrounding Antarctica (Fig. 1). Although warmer than the polar surface waters, these deeper water masses actually are denser because of their greater salinity, and consequently they sink below the surface as they approach the polar regions. The fact that such warm water masses have no surface expression in the vicinity of the ice sheets makes them impossible (thus far) to detect via remote sensing, greatly complicating the task of monitoring water-mass changes. These warm waters have a large impact where they contact glacial ice, causing melting rates of orders of tens or more meters per year (5, 6).

Accurate projections of the role of ocean-induced ice-sheet melting require improved models of the variability in circulation of CDW and IW/AW where they come in contact with glacial ice (Fig. 2D). From scant observations taken over the past few decades, these water masses are known to vary, often in response to fluctuations in atmospheric circulation. In particular, westerly winds blowing across the Southern Ocean and North Atlantic influence the movement and distribution of CDW and IW/AW water, respectively (8, 9). As a result, what controls shifts in the winds is an area of intense current research, and part of the answer appears to be natural variability, internal to the climate system, making projections of future change all the more challenging (8–10).

Ice Shelves

Much of Antarctica and areas of northern Greenland discharge ice through large floating ice shelves (Fig. 3). Once afloat, ice largely completes its direct contribution to sea level, but ice shelves exposed to oceanic melting (Fig. 1) and other climatic processes still play an important role in regulating ice-sheet discharge. With no ice shelf, the column-integrated pressure difference across the ice-ocean boundary produces a seaward-directed force at the grounding line, which is resisted upstream by friction at the ice-bed interface (11). The ocean provides negligible traction, so an unconfined (i.e., ocean-only contact) ice shelf does not alter this stress distribution. Where an ice shelf is confined by an embayment or pinning points, however, the resulting drag produces ice-shelf buttressing that offsets some of the traction that the grounded ice upstream would otherwise provide (11, 12). Thus, any loss of this buttressing from ice-shelf shrinkage or breakup must in turn be compensated for by increased drag from upstream. For the nonlinear viscous flow of ice, this restoration is achieved through increased strain rates (i.e., faster flow) (11, 13–16).

The bedrock geometry on which an ice sheet rests provides an important control on its stability. With no ice-shelf buttressing, ice discharge scales nonlinearly ($n > 3$) with grounding-line thickness, making it difficult to stabilize the grounding line on slopes where the bed deepens with distance inland (7). Thus, for extended regions where ice rests on a bed that is both well below sea level and deepens toward the interior, this nonlinearity leads to the so-called marine ice-sheet instability (17). Stabilizing factors such as ice-shelf buttressing and locally interior-shallowing slopes have allowed the predominantly marine West Antarctic Ice Sheet to maintain a relatively stable geometry even while sustaining moderate regional retreat over the past several millennia (18, 19). Ice-ocean interaction now appears to be accelerating retreat and ice-sheet loss with consequent impact on sea level.

Ice-shelf cavities generally can be classified as "warm," where water well above the local

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freezing point (e.g., CDW) flows beneath to cause strong (tens of m per year) melting, and as “cold,” where such water is largely absent. With little heat introduced into cold cavities, relatively slow (centimeters to a few m per year) melting is primarily controlled by thermohaline processes related to sea-ice formation and by tidal mixing near the ice-shelf front, as illustrated in Fig. 1 (6). Little thinning is observed today on most ice shelves with cold cavities (Fig. 3), and some are thickening substantially (20). In contrast, most ice shelves with warm cavities are thinning (Fig. 3), and nearly all of these correspond to areas where the interior ice sheet also is thinning (21, 22). Such thinning is strongest along Antarctica’s Amundsen coast, where models and data indicate that recent shifts in the Amundsen Sea Low and an atmospheric Rossby wave response to tropical warming have caused enhanced offshore Ekman transport, increasing inflow of CDW to ice-shelf cavities (9, 10) (Fig. 2C) and producing ~50% greater melt relative to the mid-1990s (23). Although a relatively strong correspondence between increased CDW flow and thinning of floating and grounded ice has been established observationally (23, 24), less is known about the precise sensitivity of ice flow to the warmer waters and the full suite of processes and feedbacks contributing to thinning.

In the Antarctica Peninsula, glaciers have responded dramatically to the loss of buttressing as ice shelves have collapsed, which has largely been attributed to warmer atmospheric temperatures and surface melting (14, 15). Along the Amundsen coast where the largest Antarctic contributions to sea level originate, the ice shelves have remained

largely intact, while grounded ice flow has accelerated. In such regions, strong thinning (up to 10 m/year; Fig. 3) of thick (hundreds of meters) ice shelves likely has reduced buttressing by at most a few percent annually (22), which is too little to directly explain much of the observed speed-up. Instead, such gradual loss of buttressing may initiate a series of feedbacks, leading to far greater losses. For example, gradual loss of buttressing may induce moderate thinning and grounding-line retreat, removing basal traction to cause further speed-up, thinning, and grounding-line retreat, especially where the bed is prone to marine ice-sheet instability (7). In response, faster speeds may cause rifts (fracturing of the full ice thickness) or crevassing along ice-shelf margins, further reducing buttressing (11, 16, 25). Lastly, as the grounding line retreats, more ice is subjected to warm ocean temperatures (24).

Although we now have a reasonable understanding of the qualitative aspects of the ocean’s interaction with large ice shelves and its key role in governing ice-sheet stability, the more quantitative understanding necessary to project sea level remains elusive. On the ocean side, high-resolution models are just beginning to resolve the details of circulation in ice-shelf cavities (26), which have largely remained unresolved at the scale of global climate models (GCMs). Recent work that does couple GCMs to regional-scale ice-ocean simulations suggests that the state-of-Texas-sized Filchner-Ronne Ice Shelf could transition from cold to warm by the end of the century, increasing basal melting by more than an order of magnitude (26). Such a change likely would lead to the ice shelf’s rapid demise, which would

remove buttressing and greatly increase outflow from several ice streams, potentially removing large marine portions of the West Antarctic Ice Sheet (27).

Once models providing accurate simulation and projection of ocean forcing are developed, they need to be coupled to ice-flow models to determine the ice-sheet response. Here, several obstacles remain. For example, grounding-line migration remains a challenge for numerical models (28); models tend to produce similar retreat but at vastly different rates (7). Much research has been discipline-focused, with rigorous coupling of ice-sheet, ocean, and climate models for long-term projection only recently becoming an area of active investigation. Until the completion of such fully coupled models that include realistic climate forcing, accurate projection of Antarctica’s contribution to sea level will be highly uncertain.

Tidewater Glaciers

Although there are a few floating ice shelves at high northern latitudes, most ice discharge from Greenland occurs through narrow, fast-moving tidewater glaciers that calve directly to the ocean from termini with little (few km) or no floating extent (Fig. 3). Over the past two decades, many of these glaciers have sped up by more than 50% (2, 29), coinciding with and likely linked to warmer waters in their respective fjords and nearby offshore locations (30–33) (Fig. 2D).

Both observation and modeling indicate that increases in speed include a major response to the loss of grounded and floating ice as glacier termini have retreated (34, 35). Similar to loss of ice-shelf buttressing, retreat of a grounded terminus

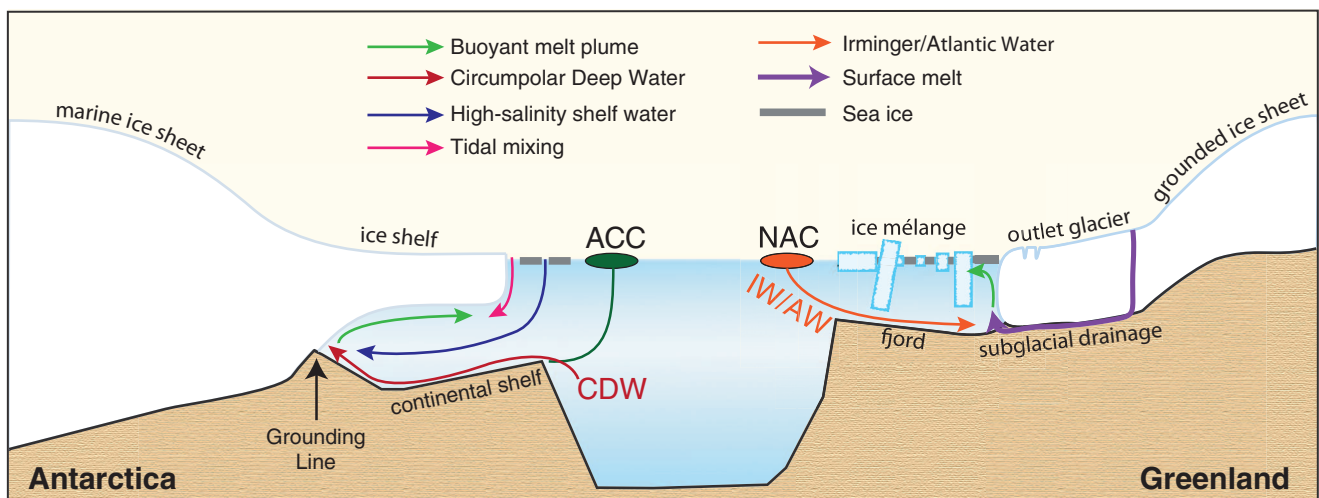


Fig. 1. Schematic illustration of the ocean processes influencing ice shelves and outlet glaciers that are described in the main text. Melting beneath Antarctic ice shelves occurs through a combination of three processes (6). The first occurs where dense, high-salinity shelf water is formed near the ice-shelf front during winter sea-ice growth. Although this water is at the surface freezing point, it can melt ice when it sinks to depths because it is above the local pressure melting point. The second occurs where tidal mixing moves seasonally warmed near-surface ocean water beneath the shelf front. Both of these processes are active for ice shelves with cold cavities. In contrast

for warm ice shelf cavities, melting is dominated by the presence of a subsurface, warm water mass (CDW), originating from the Antarctic Circumpolar Current (ACC). Ocean melting of Greenland outlet glaciers is driven by analogous warm waters, namely the IW along the western and southeastern coasts and AW elsewhere. Both of these subsurface water masses originate with the North Atlantic Current (NAC). Where melting occurs, the buoyancy of the resulting meltwater plume produces positive feedback driving further melt, which may be enhanced further where subglacial meltwater is present (38).

removes downstream resistive stresses (e.g., ice-bed and sidewall traction) that must be redistributed upstream via increased strain rates (faster flow). Once a retreat begins, additional positive feedbacks such as steepening of surface slopes and reduction in effective pressure (the excess of the weight of the ice over basal water pressure) as ice thins may amplify the response (36).

How the ocean triggers initial retreat of a tide-water glacier front, with little or no ice shelf, is still poorly understood, but several potential contributing processes have been identified, many of which relate to enhanced melt (37). Where warmer ocean waters reach glacier termini, summer melt may occur at rates of up to 3 to 4 m/day (38, 39). Rapid melting of ice cliffs requires vigorous turbulent flows to prevent formation of stable boundary layers that otherwise would partially insulate the ice from the warm ocean water. Such flows are enhanced in the summer by meltwater from the upper surface of the ice sheet, which drains to the bed to reemerge beneath the terminus and then rise buoyantly as depicted in Fig. 1 (38). Such meltwater-driven plumes also may affect any floating ice tongues that develop in regions of rapid surface melting. Thus, although water temperatures in fjords may vary little seasonally (31), melt rates may approximately double in the summer when driven by freshwater runoff (38). It is important to note that many fjords are choked with a mélange of sea ice and icebergs (Fig. 4) that can extend deep (>100 m) into the water column. Melting of such ice must consume

some poorly known fraction of the oceanic heat that might otherwise produce melt at the terminus.

The degree to which melt influences a glacier depends on the extent to which its geometry exposes it to the ocean. For example, in western Greenland in the early 2000s (see location in Fig. 2), the collapse of Jakobshavn Isbræ's ~15-km-long floating ice tongue may have been the result of thinning brought on by high (~1 m/day) melt rates (30, 40). In contrast, where glacier termini are grounded with little or no floating extension, far less area is exposed to warm waters. In such cases, melt rates (up to 4 m/day) are moderate relative to mean calving rates (~10 to 40 m/day). Whereas increased melting (by ~1 m/year) could cause gradual retreats of up to a few hundred meters per year, much of the observed retreat has occurred rapidly in association with enhanced calving rather than by gradual melt back (35, 41). In such cases, increased melt may play a role in producing excess calving, perhaps through melting from tidally driven flow of warm water beneath the ice.

Another way that warmer fjord waters may contribute to glacier retreat and speed-up is through the influence of melt on the ice mélange choking many glacier fjords (Fig. 4), especially in winter. In summer, the mélange's constituent icebergs typically float freely, but in winter sea ice bonds them together to form a rigid mass that is pushed down the fjord by the advancing glacier terminus (42). Although the strength of this amalgam should have only a small effect on the

back stress, observation and theory both indicate that it can suppress wintertime calving (42, 43), which picks up again in spring and summer when the mélange remobilizes and clears the fjord. On Jakobshavn Isbræ, this seasonal variation of calving rates allows the terminus to advance over the winter and retreat during the summer, causing its speed to vary annually by 20 to 30% near the terminus. Warmer water in the fjord may shorten the period when the mélange is frozen, causing retreat by lengthening the duration of when strong calving occurs. For example, the breakup of Jakobshavn Isbræ's ice tongue coincided with a period of reduced sea-ice concentration in nearby Disko Bay (42).

Although warmer ocean temperatures likely influence glacier stability through the processes just described, the extent to which each of these or other unidentified processes contribute remains poorly characterized. Collecting oceanic observations near the calving front presents a major challenge, because at any moment skyscraper-size blocks could tumble over or rise from below, releasing seismic energy equivalent to a magnitude-5.0 earthquake (44). Furthermore, much of the area is perennially ice covered, and deep-draft icebergs scour fjord bottoms, making it difficult to emplace oceanographic instruments. Such factors have greatly impeded progress. As a result, a solid causal understanding of the strong correspondence between warmer ocean and atmospheric temperatures around Greenland and glacier retreat, calving, and acceleration is lacking, presenting a

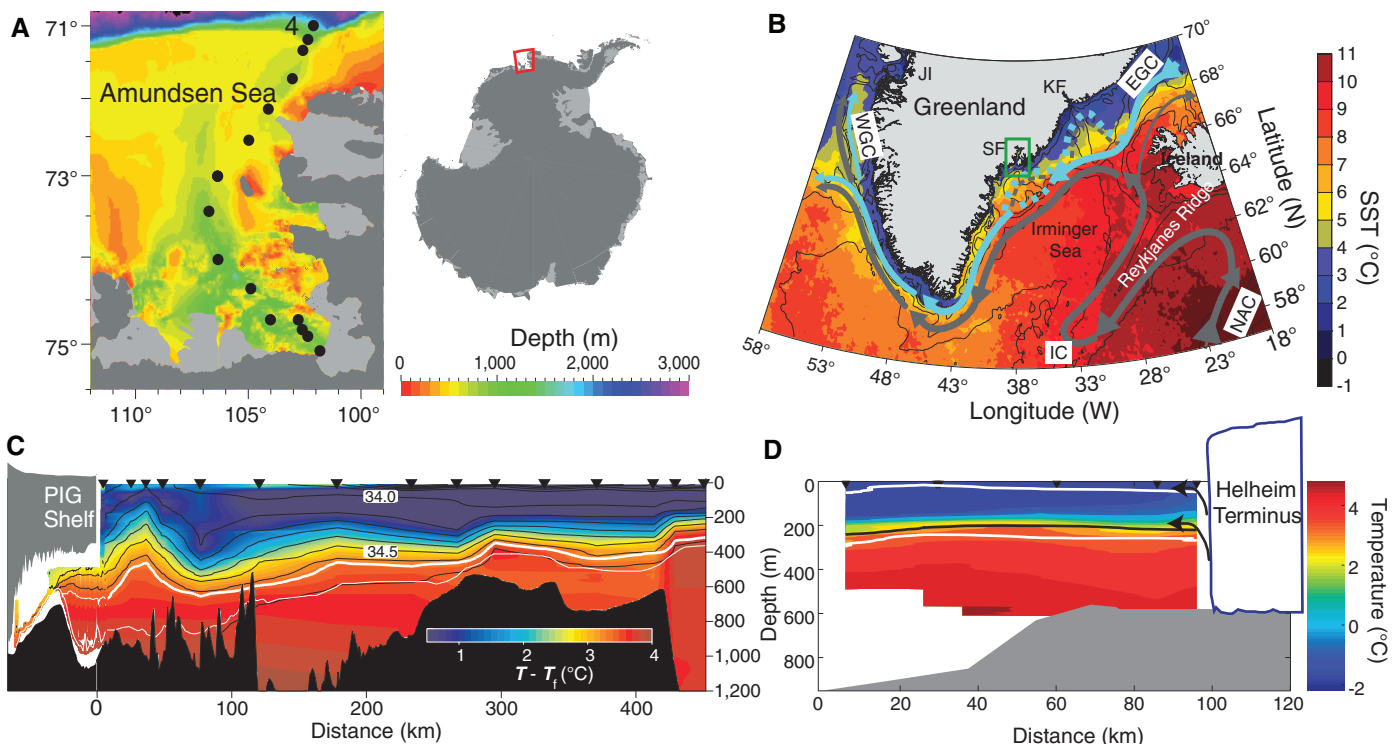


Fig. 2. Locations of temperature observations from (A) the Amundsen Sea, Antarctica, and (B) Sermilik Fjord (SF), Greenland. Also shown are the locations of Greenland's three largest glaciers along with their respective fjords: Helheim (SF), Kangerdlugssuaq (KF), and Jakobshavn Isbræ (JI). Color indi-

cates water temperature (different scale for each location) in the proximity of (C) Pine Island Glacier and (D) Helheim Glacier. [Credit: Adapted by permission from Macmillan Publishers Limited: *Nature Geosciences* (23, 31, 32), copyright (2010 and 2011)].

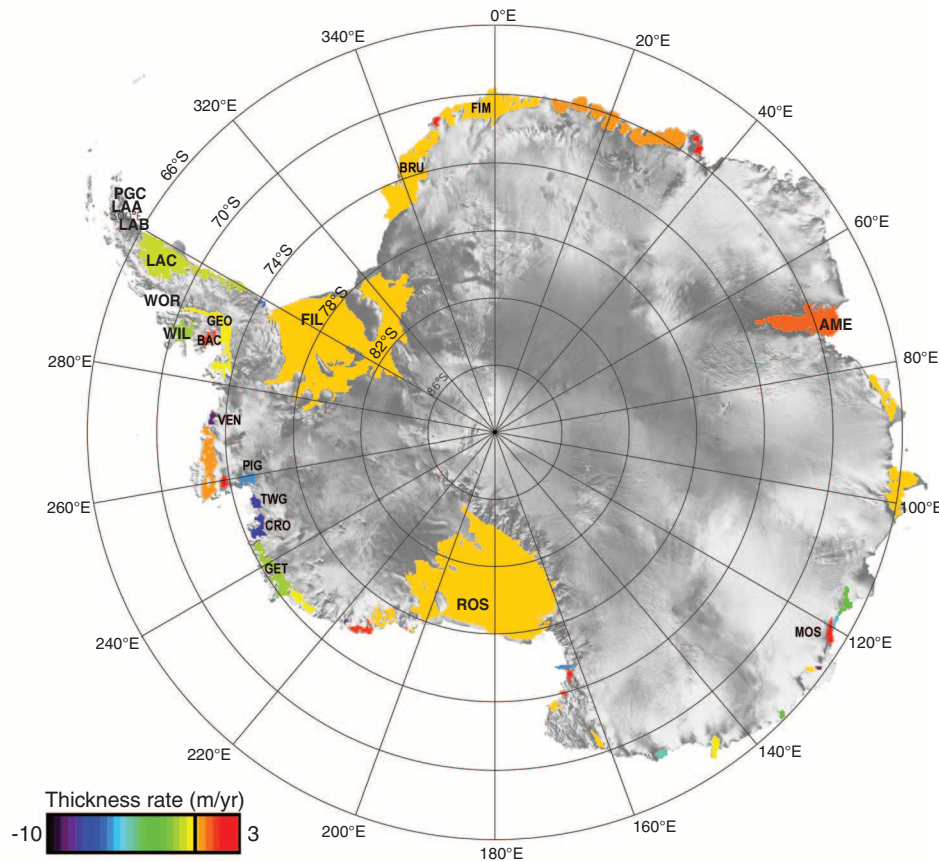


Fig. 3. Rate of thickness change of Antarctic ice shelves, with red indicating growth; green-purple, shrinkage; and yellow, stability. The rates were derived by Shepherd *et al.* (20) using radar altimetry. AME, Amery; MOS, Moscow University; ROS, Ross; GET, Getz; CRO, Crosson/Dotson; TWG, Thwaites Glacier; PIG, Pine Island Glacier; VEN, Venable Ice Shelf; FIL, Filchner; GEO, George IV; BAC, Bach; WIL, Wilkins; WOR, Wordie; LA A to C, Larsen A to C ice shelves; and PGC, Prince Gustav Channel. [Credit: Copyright 2010 American Geophysical Union (20). Reproduced by permission of American Geophysical Union.]

major interdisciplinary research challenge. Future progress likely will rely on the development of a new suite of innovative oceanographic and glaciological instrumentation, with an emphasis on automated and smart-sensing capabilities notably beyond what is currently available.

Paleo Perspective

The history of ice-sheet fluctuations confirms the strong influence of oceanic conditions on land-ice extent and volume. In particular, advance and retreat of the Greenland and Antarctic ice sheets appear to have been driven in important ways by the ocean, which at times may have been more important than atmospheric forcing. In Antarctica, it is clear that surface melting played almost no role in advance and retreat, and ice loss occurred as precipitation increased. Furthermore, differences in behavior between marine and terrestrial margins suggest that surface melting was not the only control in Greenland [e.g., (45)].

Sea level exerts an oceanic control on ice sheets. As often assumed, growth and shrinkage of the North American and Eurasian ice sheets may have contributed to long-term changes in Antarctica

through sea-level-forced shifts in grounding-line position. Sea-level forcing, however, is generally quite slow compared with potential rates of forcing by warmer water [e.g., (46)], and several feedbacks tend to stabilize ice margins against rising sea level. One such feedback is sedimentation at grounding lines, which often can keep up with rates of sea-level rise (46). Furthermore, thinning of grounded ice in response to sea-level rise reduces the gravitational attraction of the ice for ocean water, as well as unloading the crust to cause isostatic rebound, reducing or reversing local sea-level rise (47). For example, extensive late-glacial raised beaches around Greenland document relative land uplift despite global sea-level rise (48). Thus, continued retreat during periods with local sea-level fall suggests that neither global nor local sea level was dominant in controlling the Greenland Ice Sheet's size. Similarly, trends in Greenland measured by Global Positioning Systems and other methods reveal little recent response to global sea-level rise (49). Thus, paleoclimatic data suggest that ice-sheet margins may have responded primarily to changes in ocean temperature or circulation.

Sea-floor sedimentary records on the Greenland and Antarctic continental shelves document a range of retreat behaviors from the Last Glacial Maximum that may involve ice-ocean interaction. The ice-flow response to such oceanic forcing likely is complex and may exhibit threshold behavior, with similar forcing producing very different short-term behavior even for rather similar ice margins (46). For example in some places, steady retreat left limited deposits that form numerous small moraines, which may be annual. Elsewhere, long-term stability (decades to millennia?) created large grounding-zone sedimentary wedges, punctuated by very rapid retreat that left essentially no grounding-zone deposits [e.g., (50)]. Retreat styles varied in different parts of Antarctica's Ross Sea [e.g., (51)] and among Greenland's drainages [e.g., (52)]. For example, adjacent drainages in the Ross Sea produced different numbers of grounding-zone wedges between intervals of rapid retreat (53).

A likely way that ice-ocean interaction has contributed to some threshold behavior is through its impact on ice-shelf thickness and extent. For example, warming-induced thinning of Jakobshavn Isbræ's floating ice tongue eventually led to its complete loss and faster terminus flow with no sustained regrowth of the tongue (42), despite the fact that the potential melting rates even with Imminger Water in the fjord would allow persistence of a reduced ice shelf capable of at least some buttressing. This is consistent with expectations from at least one proposed ice-berg calving law (54) and suggests hysteresis with easier retreat than advance (55). The sedimentary record indicates that such behavior has occurred in the past, with an odd set of ridges in Pine Island Bay, Antarctica, likely recording the break-off of an extensive ice shelf during retreat across the continental shelf (56).

The enigmatic Heinrich events of the North Atlantic also may document ice-shelf break-off resulting from ice-ocean interaction. Because of the near-ubiquitous basal melting close to grounding lines, ice shelves tend to serve as debris filters, delaying (decades to centuries) ice-berg formation while some or all of the entrained debris is melted free and deposited beneath the shelf (57). Accordingly, the sudden onset of extensive ice-rafted debris at distant locations such as occurred during Heinrich events suggests ice-shelf loss. Under this hypothesis, a rapid ice-shelf breakup, perhaps in response to warming of waters in the sub-ice-shelf cavity, creates icebergs from debris-rich areas near the grounding line that drift with much of their debris-load intact to the North Atlantic (58).

Although far from complete, the paleoclimatic record offers numerous indications that ocean-ice interaction has been important over the longer term and probably dominant in controlling marginal fluctuations of ice sheets through glacial



Fig. 4. Ice mélange (middle of photo) in front of the roughly 10-km-wide calving terminus of Jakobshavn Isbræ (see Fig. 2 for location), which lies between the two rock outcrops (just above the middle of the photo). Until the early 2000s, a several-hundred-meter-thick ice tongue existed in this now ~14-km-long mélange-covered area. [Photo credit: I. Joughin]

cycles as well as in present times. Gradual retreats have been observed, but stability punctuated by rapid retreat has been common, with hysteresis likely occurring, much as may be the case for many recent changes.

Outlook

Numerous observations indicate that ice-ocean interaction drives much of the recent increase in mass loss from both the Greenland and Antarctic ice sheets. Much less well understood are the details of the processes by which warmer waters in contact with glacial ice lead to greater ice loss, particularly for tidewater glaciers in Greenland. Although ice-ocean processes are at least qualitatively better understood in Antarctica, there are still numerous challenges to understanding the ice-sheet response to warmer ocean waters, in particular problems related to grounding line migration (28) and iceberg calving. Furthermore, little is known about future ocean forcing. Physical understanding indicates that bulk ocean warming would speed ice loss, but the recent observed changes have been driven less by such warming than by transport of already warm water into ice-shelf cavities and fjords with dimensions well below the resolution of most GCMs, indicating the need for finer-scale regional models. Where regional-scale ocean models have been coupled to GCMs, the results indicate the potential for far more extreme changes within this century than had been anticipated (26).

Although large uncertainties remain, tremendous progress has been made over the past two decades in identifying the role ice-ocean interaction plays in ice-sheet stability. The remaining challenges require a coordinated and sustained effort by glaciologists, oceanographers, and climate modelers before reliable projections of future sea level can be made. Until that time, Greenland and

Antarctica will remain the “wild cards” in sea-level projections.

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Three-Dimensional Structures Self-Assembled from DNA Bricks

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We describe a simple and robust method to construct complex three-dimensional (3D) structures by using short synthetic DNA strands that we call “DNA bricks.” In one-step annealing reactions, bricks with hundreds of distinct sequences self-assemble into prescribed 3D shapes. Each 32-nucleotide brick is a modular component; it binds to four local neighbors and can be removed or added independently. Each 8–base pair interaction between bricks defines a voxel with dimensions of 2.5 by 2.5 by 2.7 nanometers, and a master brick collection defines a “molecular canvas” with dimensions of 10 by 10 by 10 voxels. By selecting subsets of bricks from this canvas, we constructed a panel of 102 distinct shapes exhibiting sophisticated surface features, as well as intricate interior cavities and tunnels.

Self-assembly of nucleic acids (DNA and RNA) provides a powerful approach for constructing sophisticated synthetic molecular structures and devices (1–31). Structures have been designed by encoding sequence complementarity in DNA strands in such a manner that by pairing up complementary segments, the strands self-organize into a prescribed target structure under appropriate physical conditions (1). From this basic principle, researchers have created diverse synthetic nucleic acid structures (27–30) such as lattices (4, 6, 8–10, 25), ribbons (15), tubes (6, 15, 25, 26), finite two-dimensional (2D) and 3D objects with defined shapes (2, 9–11, 13, 16–19, 22, 23, 26), and macroscopic crystals (20). In addition to static structures, various dynamic systems have been constructed (31), including switches (5), walkers (7, 14, 21), circuits (12, 14, 24), and triggered assembly systems (14). Additionally, because DNA and RNA can be interfaced with other functional molecules in a technologically relevant fashion, synthetic nucleic acid structures promise diverse applications; researchers are using nucleic acid structures and devices to direct spatial arrangement of functional molecules (6, 25, 32–34), facilitate protein structure determination (35), develop bioimaging probes (33, 34), study single-molecule biophysics (36), and modulate biosynthetic and cell-signaling pathways (25, 37).

An effective method for assembling megadalton nanoscale 2D (11) and 3D shapes (16–19, 23) is

DNA origami (29), in which a long “scaffold” strand (often a viral genomic DNA) is folded to a predesigned shape via interactions with hundreds of short “staple” strands. However, each distinct shape typically requires a new scaffold routing design and the synthesis of a different set of staple strands. In contrast, construction from standardized small components (such as DNA tiles) that each can be included, excluded, or replaced without altering the rest of the structure—modular assembly—offers a simpler approach to constructing shapes. In addition, if all components are short strands that can be chemically synthesized, the resulting structures would have greater chemical diversity than DNA origami, which typically contains half biological material (the scaffold) in mass and half synthetic material (the staples). A variety of structures have been assembled by using DNA (3, 4, 6, 8, 10, 13, 15, 20) and RNA (9, 22, 25) tiles, including periodic (4, 6, 25) and algorithmic (8) 2D lattices, extended ribbons (15) and tubes (6, 15, 25), 3D crystals (20), polyhedra (13, 22), and finite 2D shapes (9, 10). However, modular self-assembly of finite-sized, discrete DNA structures has generally lacked the complexity that DNA origami can offer.

Only recently have researchers demonstrated finite complex 2D shapes (26) self-assembled from hundreds of distinct single-stranded tiles (SSTs) (15). Unlike a traditional multistranded tile (3, 4, 6, 8–10, 13, 20, 25), which is a well-folded, compact structure displaying several sticky ends, an SST is a floppy single-strand DNA composed entirely of concatenated sticky ends. In one-pot reactions, hundreds of SSTs self-assemble into desired target structures mediated by inter-tile binding interactions; no scaffold strand is required. The simplicity and modularity of this approach allowed the authors to build more than 100 distinct shapes by selecting subsets of tiles from a common 2D “molecular canvas.” This latest success has challenged previous thinking that modular components, such as DNA tiles, are not suitable for assembling complex, singu-

larly addressable shapes (38). This presumption was largely based on a supposed technically challenging requirement for perfect strand stoichiometry (the relative ratio of the strands). Deviations from equality were expected to result in predominating partial structure formation (38). The surprising success of SST assembly may have bypassed this challenge via putative slow and sparse nucleation followed by fast growth (26), so that a large number of particles complete their formation well before depletion of the component strand pool.

Here, we generalize the concept of single-stranded “tiles” to “bricks” and thus extend our modular-assembly method from 2D to 3D. A canonical DNA brick is a 32-nucleotide (nt) single strand with four 8-nt binding domains (sticky ends). In simple one-step annealing reactions, prescribed target 3D structures self-assemble robustly from hundreds of unpurified brick strands that are mixed together with no tight control of stoichiometry. The modularity of our method enabled the construction of 102 distinct structures by simply selecting subsets of bricks from a common 3D cuboid molecular canvas consisting of 1000 voxels (fig. S1) (39); each voxel fits 8 base pairs (bp) and measures approximately 2.5 by 2.5 by 2.7 nm. These structures include solid shapes, with sophisticated geometries and surface patterns and hollow shapes, with intricate tunnels and enclosed cavities. Additionally, we have constructed structures with alternative packing geometries or using noncanonical brick motifs, demonstrating the method’s versatility. The work here thus establishes DNA bricks as a simple, robust, modular, and versatile framework for constructing complex 3D nanostructures by using only short synthetic DNA strands. More generally, it demonstrates how complex 3D molecular structures can be assembled from small, modular components mediated strictly by local binding interactions.

Design of DNA-Brick Structures and a 3D Molecular Canvas

In our design, a DNA brick is a 32-nt strand that we conceptualize as four consecutive 8-nt domains (Fig. 1A). Each DNA brick bears a distinct nucleotide sequence. All DNA bricks adopt an identical shape when incorporated into the target structure: two 16-nt antiparallel helices joined by a single phosphate linkage. The two domains adjacent to the linkage are designated as “head” domains, and the other two are designated as “tail” domains. A DNA brick with a tail domain bearing sequence “a” can interact productively with a neighboring brick with a complementary “a*” head domain in a stereospecific fashion. Each pairing between bricks defines three parallel helices packed to produce a 90° dihedral angle (Fig. 1B, top); this angle derives from the approximate 3/4 right-handed helical twist of 8 bp of DNA.

We introduce a LEGO-like model to depict the design in a simple manner (Fig. 1B, bottom). The model intentionally overlooks the detailed helical

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structure and strand polarity but preserves the aspect ratios and some of the orientational constraints on interactions between DNA bricks: The two protruding round plugs, pointing in the same direction as the helical axes, represent the two tail domains; the two connected cubes with recessed round holes represent the two head domains. A brick must adopt one of two classes of orientation, horizontal or vertical (Fig. 1B). The two bricks connect to form a 90° angle via hybridization, represented as the insertion of a plug into a hole. An insertion is only allowed between a plug and a hole that carry complementary sequences with matching polarity (which is not graphically depicted in the current model for expositional simplicity). In fig. S2, we present a more detailed LEGO-like model that explicitly tracks the polarity of the DNA bricks and their stereospecific interaction pattern.

Structural periodicities of the design are illustrated in a 6H (helix) by 6H (helix) by 48B (bp) cuboid structure (Fig. 1, C and D). Bricks

can be grouped into 8-bp layers that contain their head domains. Bricks follow a 90° counterclockwise rotation along successive 8-bp layers, resulting in a repeating unit with consistent brick orientation and arrangement every four layers. For example, the first and fifth 8-bp layers in Fig. 1D share the same arrangement of bricks. Within an 8-bp layer, all bricks share the same orientation and form a staggered arrangement to tile the layer. On the boundary of each layer, some DNA bricks are bisected to half-bricks, representing a single helix with two domains. The cuboid is self-assembled from DNA bricks in a one-step reaction. Each brick carries a particular sequence that directs it to fit only to its predesigned position. Because of its modular architecture, a predesigned DNA brick structure can be used for construction of smaller custom shapes assembled from subsets of DNA bricks (Fig. 1E). Detailed strand diagrams for the DNA brick structures are provided in figs. S3 and S4.

3D molecular canvas. The LEGO-like model can be further abstracted to a 3D model that contains only positional information of each 8-bp duplex. A 10H by 10H by 80B cuboid is conceptualized as a 3D molecular canvas that contains 10 by 10 by 10 voxels. Each voxel fits an 8-bp duplex and measures 2.5 by 2.5 by 2.7 nm (Fig. 1F). Based on the 3D canvas, a computer program first generates a full set of DNA bricks, including full-bricks and half-bricks that can be used to build a prescribed custom shape. Using 3D modeling software, a designer then needs only to define the target shapes by removing unwanted voxels from the 3D canvas—a process resembling 3D sculpting. Subsequently, the computer program analyzes the shape and automatically selects the correct subset of bricks for self-assembly of the shape.

Self-Assembly of DNA-Brick Cuboid Structures

Using the above design strategy, we constructed a wide range of DNA brick structures (39). We

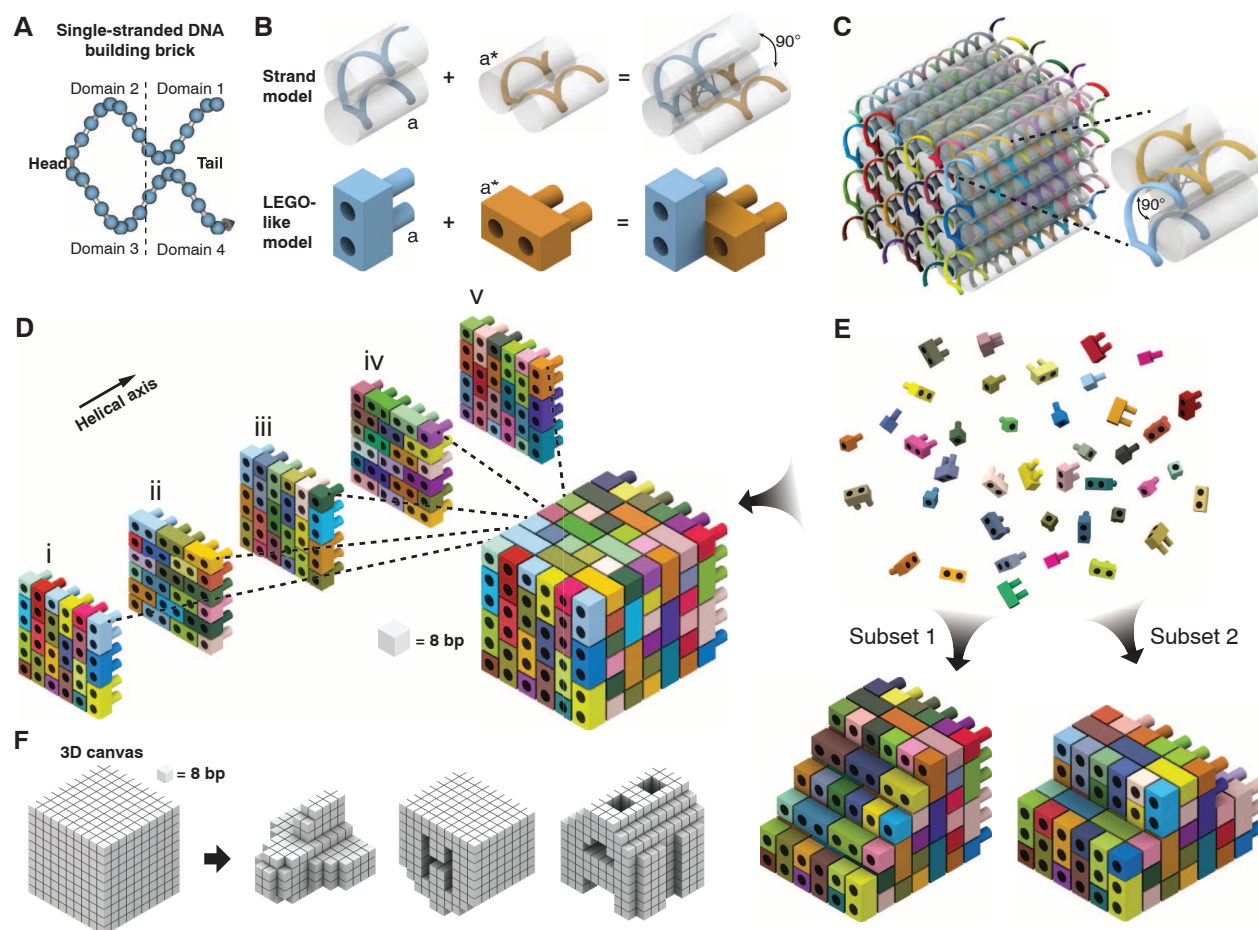


Fig. 1. Design of DNA brick structures analogous to structures built of LEGO® bricks. (A) A 32-nt four-domain single-stranded DNA brick. Each domain is 8 nt in length. The connected domains 2 and 3 are “head” domains; domains 1 and 4 are “tail” domains. (B) Each two-brick assembly forms a 90° dihedral angle via hybridization of two complementary 8-nt domains “a” and “a*.” (C) A molecular model that shows the helical structure of a 6H by 6H by 48B cuboid 3D DNA structure. Each strand has a particular sequence, as indicated by a distinct color. The inset shows a pair of bricks. (D) A LEGO-like model of

the 6H by 6H by 48B cuboid. Each brick has a particular sequence. The color use is consistent with (B). Half bricks are present on the boundary of each layer. (E) The 6H by 6H by 48B cuboid is self-assembled from DNA bricks. The bricks are not interchangeable during self-assembly because of the distinct sequence of each brick. Using the 6H by 6H by 48B as a 3D molecular canvas, a smaller shape can be designed by using a subset of the bricks. (F) 3D shapes designed from a 10 by 10 by 10-voxel 3D canvas; each voxel fits 8 bp (2.5 nm by 2.5 nm by 2.7 nm).

first constructed 3D cuboid structures of a variety of sizes and aspect ratios (Fig. 2).

Random sequence design. The sequences of DNA bricks were designed by random assignments of base pairs (A-T, C-G) to 3D structures. We first tested two versions of a 6H by 6H by 64B cuboid, with either random sequences or specially designed sequences (designed by smoothing binding energy, minimizing undesired secondary structure, and reducing sequence symmetry) and observed comparable self-assembly yields (fig. S5). We also tested three sets of random sequences using a 4H by 12H by 120B cuboid and again observed similar assembly yields (figs. S6 and S7; more discussion on domain similarity of random sequence design is provided in fig. S8). Thus, random sequences were applied to all subsequent designs.

Protector bricks. Including unpaired single strands at the ends of DNA duplexes has proven to be effective for mitigating unwanted aggregation that results from blunt-end stacking (11). An 8-nt single-stranded domain protruded out from every 5' or 3' end of all DNA duplexes in our 3D structure designs (Fig. 1C). The sequences of these 8-nt domains were replaced with eight continuous thymidines to further prevent undesired nonspecific binding interactions between exposed single-stranded domains. DNA bricks with modified head or tail poly-T domains are named “head protectors” or “tail protectors,” respectively.

Boundary bricks. A 16-nt half brick could be merged with a preceding 32-nt full brick along the direction of its helix to form a 48-nt strand (figs. S9 to S11). We observed a 1.4-fold improvement in assembly yield for a 6H by 6H by 64B cuboid when this 48-nt boundary-strand design was implemented, possibly reflecting accelerated nucleation of target structure formation. Hence,

this merge strategy was applied to all of our 3D structures.

Assembly and characterization of 6H by 10H by 128B cuboid. For a detailed characterization study, we constructed a 6H by 10H by 128B cuboid (Fig. 2A). It consists of 459 strands (7680 bp, with a molecular weight comparable with that of an M13-based DNA origami; design details are provided in figs. S12 and S13). Unpurified DNA strands were mixed together at nominally equal ratios without careful adjustment of stoichiometry (39). To determine the optimal assembly conditions, we tested two annealing ramps (24-hour annealing and 72-hour annealing), two strand concentrations (100 and 200 nM per strand), and eight MgCl₂ concentrations (10, 20, 30, 40, 50, 60, 70, and 80 mM). Equal amounts of each sample (2 pmol per strand) were then subjected to EtBr-stained 2% agarose gel electrophoresis (fig. S14). The best gel yield (~4% as calculated by yield = measured mass of product/mass of all strands) was achieved at the following conditions: 200 nM per strand, 72-hour annealing, 40 mM MgCl₂ (fig. S15). The above gel yield reflects only an approximate estimate for the incorporation ratio of the monomer strands (26).

For comparison, 4 to 14% gel yield was reported for 3D DNA origami with similar size and aspect ratios [such as the 10H by 6H by 98B and other origami cuboids in (40)]. The origami gel yield was estimated as yield = (scaffold strands incorporated into product/total scaffold strands); the loss of excessive staple strands (normally 5- to 10-fold more than the scaffold strand) was not taken into account. For DNA bricks, the optimal 40 mM MgCl₂ was higher than the optimal MgCl₂ concentration for 3D origami folding, which typically is below 30 mM (18). Column-purified DNA bricks product (~50% recovery

efficiency) (Fig. 2B) migrated as a single band on agarose gel and appeared under transmission electron microscopy (TEM) with expected morphology (Fig. 2C) and measured dimensions of 0.34 nm ($\pm$ 0.01 nm SD) per base pair and 2.5 nm ($\pm$ 0.2 nm SD) per helix width. For the gel-purified product, “the percentage of intact structures” was estimated at 55% by counting the ratio of intact particles over all the particles in TEM images (fig. S16). This percentage of intact structures is comparable with the previously reported percentages of 3D square-lattice DNA origami (27% for a 6H by 12H by 80B cuboid, 59% for an 8H by 8H by 96B cuboid) (41).

Special designs can be applied to increase the assembly yield of the 6H by 10H by 128B cuboid. “Head protectors” and “tail protectors” appeared especially unstable because half of their 8-nt domains are unpaired. By merging “head protectors” of the 6H by 10H by 128B cuboid with their neighboring strands (figs. S17 and S18), a modified version 6H by 10H by 128B-M cuboid was obtained and showed 190% improvement in gel assembly yield and 17% improvement in the percentage of intact structures under TEM over the standard 6H by 10H by 128B cuboid (fig. S19). Thus, 3D structures can be further stabilized by using special design rules, such as this merging strategy. However, this modification requires deletions of crossovers between helices, which may potentially create global or local deformations, and was not used for constructions in the remainder of the paper.

Structures of different sizes. Eighteen distinct cuboid structures that contain 9, 16, 36, 60, 96, and 144 helices were designed, annealed using the optimal conditions previously identified for the 6H by 10H by 128B cuboid self-assembly, and characterized through gel and TEM (Fig. 2D and

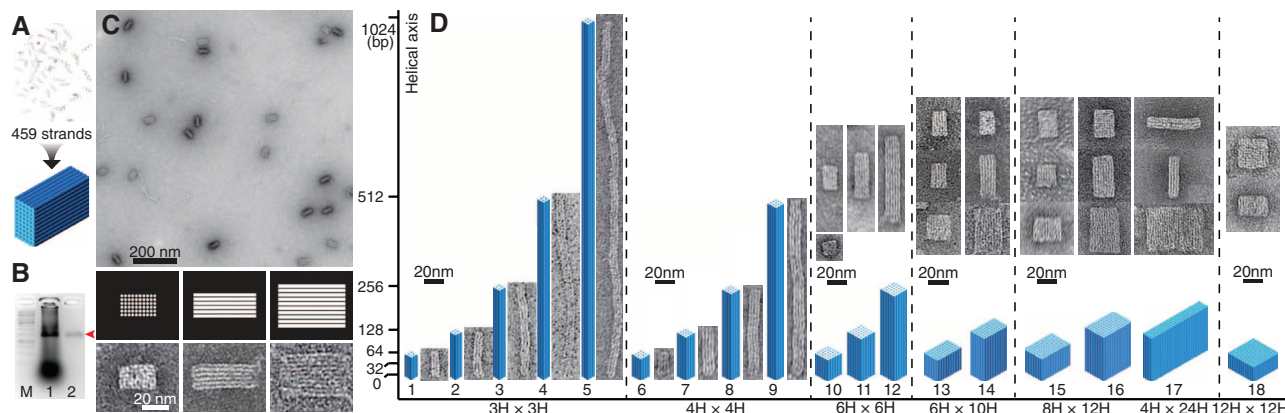


Fig. 2. Cuboid structures self-assembled from DNA bricks. **(A)** DNA bricks self-assembled into a 6H by 10H by 128B cuboid in a one-step thermal annealing process. **(B)** Agarose gel electrophoresis showing 50% purification recovery efficiency of the 6H by 10H by 128B cuboid. Lane M contains the 1-kb ladder. Lanes 1 and 2 contain unpurified and purified 6H by 10H by 128B cuboid structures, respectively. The red arrow points to the cuboid product band. **(C)** TEM images of gel-purified 6H by 10H by 128B cuboid. Zoomed-in images (bottom) and corresponding computer-generated graphics (middle) show three

different projection views. **(D)** Designs and TEM images of 18 cuboids of a variety of dimensions. Horizontal axis is labeled with the cross-section dimensions of the cuboids; vertical axis is labeled with the lengths of the constituent helices. The lengths are 48B (shape 18), 64B (shapes 1, 6, 10, 13, and 15), 120B (shapes 16 and 17), 128B (shapes 2, 7, 11, and 14), 256B (shapes 3, 8, and 12), 512B (shapes 4 and 9), and 1024B (shape 5). Each 3D cylinder model is drawn proportionally to the relative dimensions of the cuboid; corresponding TEM images are shown to the right or above each model.

fig. S20). Additional TEM images are shown in figs. S21 to S27. Measured dimensions of intact particles for each structure agree with the designs (fig. S28). Gel yields varied from <1 to ~80% (figs. S20C and S28). For structures with the same number of helices, smaller cuboids exhibited higher assembly yields. The highest yield (80%) was observed for the smallest object, the 3H by 3H by 64B cuboid; the lowest yields (<1%) were observed for the 8H by 12H by 120B, 4H by 24H by 120B, and 12H by 12H by 48B cuboids. The biggest DNA objects constructed in this paper are an 8H by 12H by 120B

cuboid (formed by 728 strands) and a 4H by 24H by 120B cuboid (formed by 710 strands), which are identical in molecular weight (24,576 nt, 8 MD, and 60% more massive than an M13-based DNA origami). Increasing the concentration for the brick strands helped to increase the yield for a small cuboid, 4H by 4H by 128B (fig. S29). In some cases, higher molecular weight bands can be detected above the product band; these bands are likely multimers caused by non-specific interactions between assembled products. For example, for the 6H by 10H by 64B structure, TEM revealed that an upper band con-

tained dimers of the cuboids (fig. S30). Cuboids with 32-bp (32B) helices were also tested but failed to assemble (fig. S20). This is likely due to the fact that these cuboids contained only one crossover between each pair of neighboring helices and hence were less stable.

Complex Shapes Made from a 10 by 10 by 10-Voxel 3D Canvas

Using the 10 by 10 by 10-voxel 3D canvas (Figs. 1F and 3A and fig. S31), we next constructed 102 distinct shapes (Fig. 3), demonstrating the modularity of the DNA brick strategy.

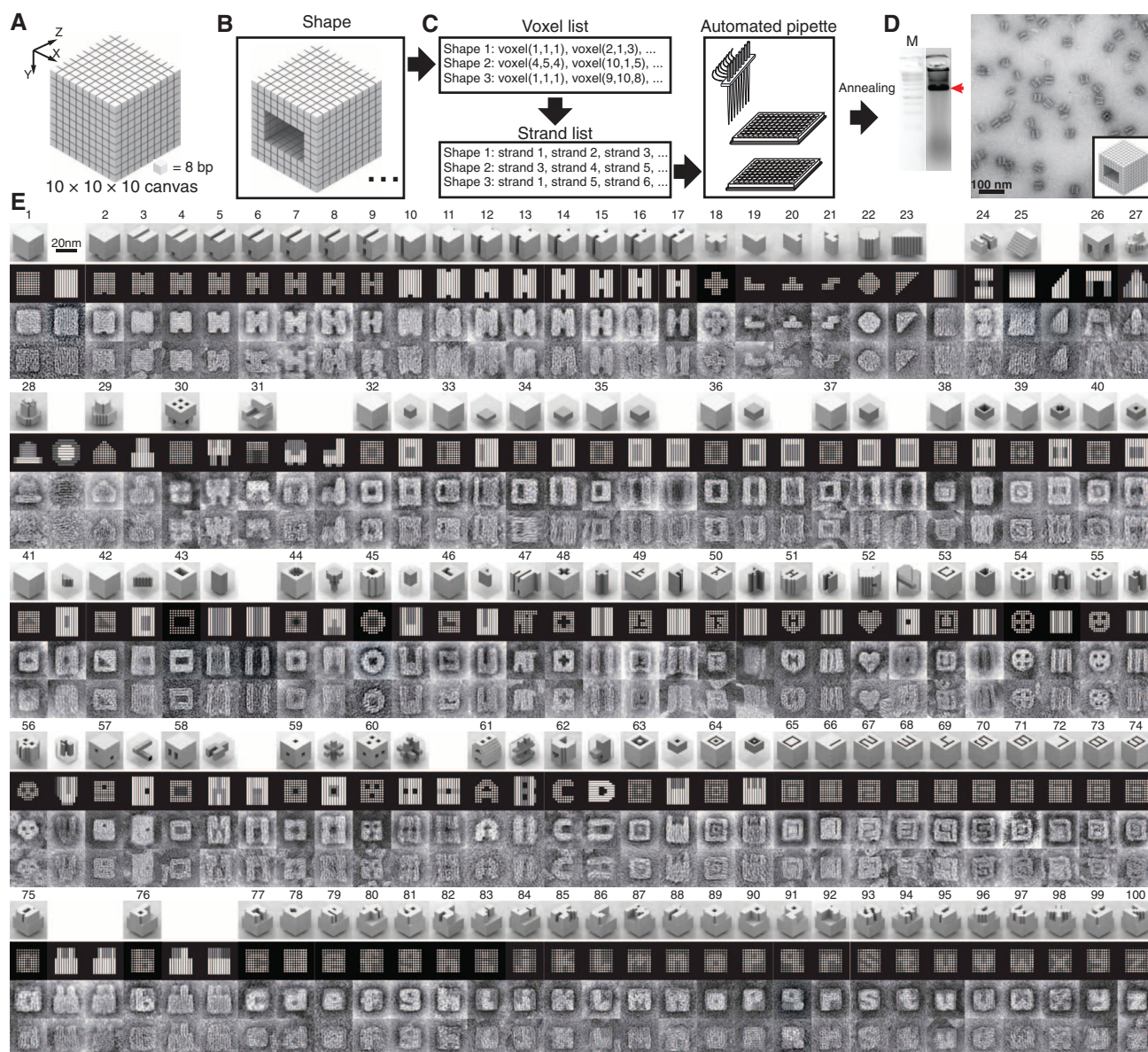


Fig. 3. Shapes made from a 3D molecular canvas. (A) A 10 by 10 by 10-voxel 3D canvas. z axis is the helical axis. Each voxel (8 bp) measures 2.5 by 2.5 by 2.7 nm. (B) Shapes are designed by editing voxels by using 3D modeling software. (C) A computer program recognizes the voxel composition of each shape and generates a list of strands to form this shape. The list then is used to direct an automated liquid-handling robot to mix the strands. (D) After annealing, the shapes are characterized by means of agarose gel electrophoresis and TEM imaging. Lane M contains the 1-kb ladder. The product band is

indicated by the red arrow. (E) Computer-generated models and TEM images of shapes. The top row for each shape depicts a 3D model, followed by a computer-generated projection view, an image averaged from six different particles visualized by using TEM, and a representative raw TEM image. More raw images are shown in figs. S38 to S54. In a number of cases, multiple projections are presented. Some shapes with cavities or tunnels are depicted with additional transparent 3D views that highlight the deleted voxels (colored dark gray). For example, the top right model of shape 32 shows the enclosed cuboid cavity.

DNA bricks and derivatives. Any brick in the 3D canvas can become either a boundary half brick (exposed at the edges of a layer and bisected) (Fig. 1D), a protector brick (located in the first or last layer along z axis), or even both at same time, in a custom shape design. Thus, modified versions of each brick were generated with all combinations of domain-deletion (bisect to a half-brick), polythymidine-sequence-substitution (change to protector bricks), and boundary-brick-merger (change to 48-nt boundary bricks) to accommodate the possibilities (two types of strands with low occurring frequency and four types of strands with only one binding domain were excluded in our implementation) (fig. S32). Overall, a master collection of 4455 strands (with a total of 138,240 nt) were generated by a computer program to guarantee that a designed shape could be assembled with head/tail protector bricks and 48-nt boundary bricks. Custom shapes were assembled via selecting subsets of the master collection without synthesizing new strands.

Automated design process. By rendering the 3D canvas using 3D modeling software, we can edit voxels and visualize a shape using a graphical user interface (Fig. 3B). Then, the voxel information of multiple shapes is interpreted by a custom program to generate a list of strands involved in the formation of each shape. This list is subsequently processed to direct an automated liquid-handling robot to select DNA strands from source plates and pipette them to the wells of a product plate, mixing strands for many shapes in a high-throughput manner (Fig. 3C). The strands will be subsequently annealed in separate test tubes to produce the desired structures (Fig. 3D). The complete design workflow is shown in figs. S33 and S34. To use existing computational tools previously developed by other researchers, we can also convert shapes to caDNA files (40). Each shape's conformation then can be simulated using CanDo (42), a software tool for computing 3D structures of DNA origami (fig. S35).

Using the 3D canvas and following the automated design process, we successfully constructed 102 distinct shapes (gels in figs. S36 and S37; TEM images of shapes 1 to 100 in Fig. 3E; and raw TEM images for all the shapes in figs. S38 to S54).

Shapes 1 to 17. The basic design constraints were studied by using a group of shapes containing two 4H by 10H by 80B blocks connected by a middle "connecting block" (shapes 2 to 17). The connecting blocks were two-voxel wide along x axis and systematically designed to possess decreasing numbers of voxels along y axis (shapes 2 to 9) or z axis (shapes 10 to 17). Eliminating voxels along the x axis should have the same effect as eliminating voxels along the y axis because of the shape symmetry. Agarose gel electrophoresis revealed that in both systems, as the connector became overly thin, the gel yields for the intact structures decreased, and partial structures (putative unconnected 4H by 10H by 80B blocks) became more prominent (for example, in lanes for shapes 8, 9, and 15 to 17 in

fig. S36). However, reducing the number of voxels along the z axis appeared to decrease the yield more significantly than along the y axis. Shape 9, which contained only a 2-voxel connection along the y axis, gave 6% gel yield. In contrast, the yield for shape 17 (2-voxel along the z axis) dropped to 1%. Overall, these observations suggest safe design criteria of at least two continuous voxels along the x axis or y axis (2 helices) and three z axis voxels (24 bp) for stable features. However, as demonstrated in following experiments, smaller features (for example, two z axis voxels, shapes 33 to 37; one x axis or y axis voxel, shapes 64 to 74) can still stably exist in certain shapes in which these features are presumably reinforced by other voxels in close proximity.

Solid shapes 18 to 31. A number of solid shapes were designed including z direction extrusions of simple geometric shapes (shapes 18 to 23) and more intricate objects (shapes 24 to 31; also, shape 102 in fig. S54). Gel yields and TEM images of these objects provided more knowledge of the design space of our methodology. For example, shapes 26 and 27, which both contained 3-helix-thick appendages anchored only on one edge, were occasionally found without these protrusions or with them but containing defects. Thus, such thin features, although obeying our design criteria, appeared to be less stable than were the better-supported or thicker features.

Closed-cavity shapes 32 to 42. Previously, a few examples of 3D DNA origami with closed cavities were demonstrated, including a box (16), a tetrahedron (17), a sphere, and an ellipsoid (23). We created a series of "empty boxes" with different sizes of cuboid cavities (shapes 32 to 37) as well as more intricate cavity shapes (such as a square ring, cross, and triangle; shapes 38 to 42).

Open-cavity shapes 43 to 62. We constructed shapes with a single open cavity (tunnel) of varying width, depth, and geometry (shapes 43 to 53) and multiple-parallel cavities (shapes 54 to 56). Shapes with noncrossing perpendicular tunnels (shape 57), turning and branching tunnels (shape 58), and crossing tunnels (shapes 59, 60; also, shape 101 in fig. S54) were also demonstrated. Furthermore, we constructed tunnel-containing cuboids with modified outer surfaces in order to create varying external views from different angles, as demonstrated by shapes 60 to 62.

Features-on-solid-base shapes 63 to 100. Sophisticated features were designed on a solid base, including a full set of 10 Arabic numerals (shapes 65 to 74) and 26 lowercase letters for the English alphabet (shapes 75 to 100). Two concentric ring structures (shapes 63 and 64) and the numerals (shapes 65 to 74) contained features as thin as one voxel (2.5 nm), suggesting that the design criteria (for example, thin structures tend to fail) are contingent on the surrounding environment of a particular feature. These shapes also highlight the capacity of creating extruded features that would otherwise be unattainable via 2D assembly (26).

For most shapes, assembly yields were between a few percent and 30% [figs. S36 and S37; in comparison, yields of five 3D DNA origami structures were reported as 7 to 44% (18)]. Only five shapes had assembly yields higher than 30%; three shapes had assembly yields lower than 1%.

In spite of our success in making a variety of intricate 3D shapes, some shapes exhibited undesired properties. For example, shapes 60 to 62 only showed <1% of intact particles in TEM images; some fine features of a shape (such as the two wings of shape 27) could be damaged or even completely missing if the shape was extracted from an agarose gel band. We also observed four failed designs that did not produce clear product bands on agarose gels (fig. S55A). Two features-on-solid-base designs showed strong bands on agarose gels (fig. S55B), and were of the expected size in TEM images. However, their features were not clearly resolved under TEM, suggesting that the shapes may have formed, but the features were too subtle to be visualized.

Generality of DNA Brick Self-Assembly

To explore the generality of the DNA brick assembly framework, we constructed structures with brick motifs other than the 32-nt canonical brick motif. These structures include those with alternative lattice geometries that have been previously demonstrated by DNA origami (11, 18, 43).

Single-layer (2D) structures. Conceptually, a single-layer structure can be constructed by "extraction" of a layer from a 3D brick structure [Fig. 4A and fig. S56, comparison with a 2D single-stranded tile rectangle design (26)]. A 30H by 1H by 126B rectangle was intentionally modified to be 10.5 bp per turn instead of 10.67 bp per turn (for 3D design) in order to get a relatively flat structure (fig. S57). Gel yield was estimated to be 18% (fig. S58), which is comparable with 2D single-stranded tile structures (26). TEM (Fig. 4B) and atomic force microscopy (AFM) (Fig. 4C) revealed expected rectangle structures. On the basis of AFM images, the dimensions were measured as 0.31 nm ($\pm$ 0.01 nm SD) per base pair and 2.6 nm ($\pm$ 0.3 nm SD) per helix width.

3D honeycomb-lattice structures. We then created 10.8-bp per turn (33.3° twist per base pair) honeycomb-lattice (HC) and hexagonal-lattice (HL) DNA structures. Four types of four-domain DNA strands were designed for HC structures (Fig. 4, D and E). A 6H by 6H by 84B-HC structure was successfully constructed and characterized (Fig. 4F and fig. S59). Particles in TEM images were measured to be 13 nm ($\pm$ 0.9 nm SD) by 22 nm ($\pm$ 1.0 nm SD) by 29 nm ($\pm$ 1.2 nm SD). Assembly yield was estimated to be 30% (fig. S60).

3D hexagonal-lattice DNA structures. Two types of strands are used to build a HL structure: a linear strand with multiple 9-nt domains and an 18-nt strand with two 9-nt domains that are connected by a crossover (Fig. 4, G and H).

A 6H by 7H by 108B-HL structure was constructed and characterized (Fig. 4I and fig. S61). Particles in TEM images were measured to be 13 nm (± 0.8 nm SD) by 18 nm (± 1.1 nm SD) by 35 nm (± 2.2 nm SD). Assembly yield was estimated to be 26% (fig. S62).

Other brick motifs. We also constructed a 6H by 10H by 64B cuboid that arranges brick strands in an alternating fashion between layers (figs. S63 and S64) and two 6H by 6H by 64B cuboids that implement two other brick motif designs (figs. S65 and S66). One design is based on “chopping” the scaffold of a DNA origami to short strands (fig. S65A). The other adopts standardized motifs that are each 32 nt long and have two crossovers (fig. S65B). These designs further demonstrate the versatility of DNA brick self-assembly.

Discussion

DNA bricks provide a simple, modular, and robust framework for assembling complex structures from short strands. Simplicity: A canonical brick is a standardized 32-nt single strand composed of four 8-nt binding domains; bricks interact via simple local binding rules. Modularity: With no scaffold present, an assembly of bricks has a modular architecture; each brick can be added or removed independently. Robustness: The assembly process is robust to variations in sequence composition (random sequences are

used), strand synthesis (unpurified strands suffice), and stoichiometry (no tight control is required). Together, the simple and standardized motif, modular architecture, and robust performance permit straightforward automation of the design and construction process. A software tool takes as input a 3D shape specification and directs a liquid-handling robot to select and mix presynthesized brick strands to form the shape. Using a 1000-voxel canvas, 102 diverse shapes were rapidly prototyped. These shapes demonstrate a new level of geometrical sophistication, as exemplified by the intricate tunnel and cavity features.

The DNA brick framework is not restricted to the canonical 32-nt motif and can be generalized to include various other motifs (Fig. 4), enabling the construction of 3D structure with diverse lattice-packing geometries. In addition, previously demonstrated single-stranded tiles (15, 26) can be viewed as a special case of bricks in which each pair of neighboring bricks form a 180° angle. For comparison, in hexagonal-, square-, and honeycomb-lattice structures, neighboring bricks form 60°, 90°, and 120° angles, respectively. These different angles are achieved by changing the domain lengths of bricks. Furthermore, neighboring bricks may be merged into a longer strand, which may facilitate nucleation or strengthen structurally weak positions. The DNA brick (and single-stranded tile) method differs from

previous multistranded tiles in that each brick monomer is a floppy single strand and only folds into a bricklike shape when incorporated into the assembly. It also differs from DNA origami by not using a scaffold strand. However, DNA origami can also be related to the brick framework, in which half of the bricks are concatenated into a long scaffold (fig. S65A). The successes of constructions that use only short strands (as in bricks) and those that include a long scaffold (as in origami) together suggest a full spectrum of motif possibilities with strands of diverse lengths: Longer strands may provide better structural support, and shorter ones may provide finer modularity and features; the eclectic use of both may lead to the most rapid progression toward greater complexity.

The DNA brick structures constructed here are still far below the size limit allowed by sequence uniqueness. Making the conservative assumption (by neglecting the contribution of cooperativity) that every domain must display a different sequence, a structure using canonical 32-nt, four-domain bricks could potentially reach a size of 8 nt by 4⁸ (524,288 nucleotides). In our experiments, the assembly process appeared to tolerate (sparse) identical domains (fig. S8), further expanding the potential obtainable size. Further exponential increase in size could potentially be achieved by using bricks with longer domains or by encoding algorithmic growth patterns (8) in

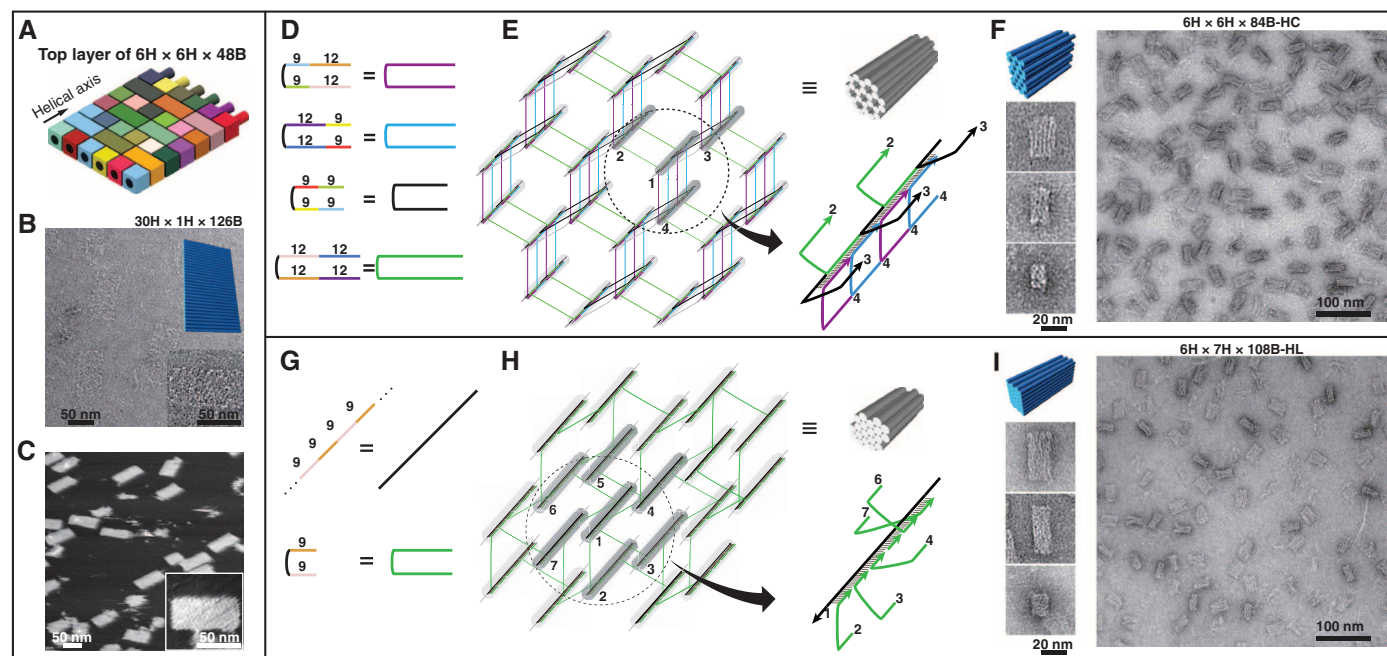


Fig. 4. Generality of DNA brick self-assembly. (A to C) The design and construction of a single-layer brick structure. (A) DNA bricks of the top layer of the 6H by 6H by 48B cuboid in Fig. 1D, with the crossovers to the layer below removed. (B) TEM images of a 30H by 1H by 126B rectangle. Top right inset shows the model of the design. Bottom right inset contains a zoomed-in image of the structure. (C) AFM images of the 30H by 1H by 126B rectangle. Inset contains a zoomed-in image of the structure. (D to I) The designs and constructions of 3D honeycomb-lattice [(D) to (F)] and hexagonal-lattice [(G) to (I)] brick structures. [(D) and (G)] The strands used

for (D) honeycomb-lattice and (G) hexagonal-lattice self-assembly. The number of nucleotides in each domain is indicated in the left panel. [(E) and (H)] Strand diagrams of (E) an 84-bp honeycomb-lattice structure and (H) a 54-bp hexagonal-lattice structure. The right bottom image depicts an enlarged image of the circled helix bundle. Strand colors match those described on the right side of (D) or (G). Numbers indicate DNA helices. [(F) and (I)] TEM images of (F) a 6H by 6H by 84B-HC hexagonal-lattice structure and (I) a 6H by 7H by 108B-HL 3D hexagonal-lattice structure. 3D model and zoomed-in images of different projection views are shown to left.

the assembly. However, in practice, low yields were already observed for larger designs (up to 24,576 nucleotides attempted thus far). Solving this challenge may require improvements in structure and sequence design, enzymatic synthesis for higher-quality strands, optimized thermal or isothermal (44) annealing conditions, and a detailed understanding and perhaps explicit engineering of the kinetic assembly pathways (8, 14, 44) of DNA brick structures.

The DNA brick structure, with its modular architecture, sophisticated geometry control, and synthetic nature, will further expand the range of applications and challenges that nucleic acid nanotechnology has already started to address—for example, to arrange technologically relevant guest molecules into functional devices (6, 25, 32–34), to serve as programmable molecular probes and instruments for biological studies (33, 34, 36), to render spatial control for biosynthesis of useful products (25), to function as smart drug delivery particles (37), and to enable high-throughput nanofabrication of complex inorganic materials for electronics or photonics applications (6, 32). The modularity of the brick structure may facilitate rapid prototyping of diverse functional nanodevices. Its sophisticated and refined geometrical control may enable applications that require high-precision arrangements of guest molecules. Because the brick structure is composed entirely of short synthetic strands (no biologically derived scaffold), it is conceivable to make bricks by using synthetic informational polymers other than the natural form of DNA. Such polymers may include L-DNA (26), DNA with chemically modified backbones or artificial bases, or chemically synthesized or in vitro (or even in vivo) transcribed RNA. This material diversity may potentially produce nanostructures with not only prescribed shapes but also designer chemical (or biochemical) properties (such as nuclease resistance or reduced immunogenicity) that would be useful for diverse applications requiring the structure to function robustly in complex environments, such as in living cells or organisms.

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Supplementary Materials

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Materials and Methods
Supplementary Text
Figs. S1 to S66
Tables S1 to S20

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A Reconciled Estimate of Ice-Sheet Mass Balance

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We combined an ensemble of satellite altimetry, interferometry, and gravimetry data sets using common geographical regions, time intervals, and models of surface mass balance and glacial isostatic adjustment to estimate the mass balance of Earth's polar ice sheets. We find that there is good agreement between different satellite methods—especially in Greenland and West Antarctica—and that combining satellite data sets leads to greater certainty. Between 1992 and 2011, the ice sheets of Greenland, East Antarctica, West Antarctica, and the Antarctic Peninsula changed in mass by -142 ± 49 , $+14 \pm 43$, -65 ± 26 , and -20 ± 14 gigatonnes year⁻¹, respectively. Since 1992, the polar ice sheets have contributed, on average, 0.59 ± 0.20 millimeter year⁻¹ to the rate of global sea-level rise.

Fluctuations in the mass of the polar ice sheets are of considerable societal importance, because they affect global sea levels (1, 2) and oceanic conditions. They occur as

a consequence of their internal dynamics and changes in atmospheric and oceanic conditions (3–5). Analysis of the geological record suggests that past climatic changes have precipitated

sustained ice-sheet contributions, in excess of 10 mm year⁻¹ over millennial time periods (6), and the prospect of such changes in the future are of greatest concern. Even the modest rises in ocean temperature that are predicted over the coming century (7) could trigger substantial ice-sheet mass loss through enhanced melting of ice shelves (8–10) and outlet glaciers (11, 12). However, these processes were not incorporated into the ice-sheet models that informed the current global climate projections (13). Until this is achieved, observations of ice-sheet mass imbalance remain essential in determining their contribution to sea level.

Satellite geodesy has revolutionized the manner in which ice-sheet mass balance is estimated (14, 15). Since 1998, there have been at least 29 ice-sheet mass balance estimates, based variously on the satellite techniques of altimetry, interferometry, and gravimetry (16). These estimates, and their respective uncertainties, allow for a combined Greenland and Antarctic ice-sheet mass im-

balance of between –676 and +69 gigatonnes (Gt) year⁻¹, equivalent to a mean global sea-level contribution in the range of +1.9 to –0.2 mm year⁻¹. However, much of this spread, which is large in comparison to other ice-sheet imbalance assessments (1, 2) and to the estimated rate of global sea-level rise (17), is due to the brevity of many satellite surveys (4.5 years, on average) relative to the rate at which ice-sheet mass fluctuates (5, 18–20). Because the various satellite methods differ in their strengths and weaknesses (14, 15, 21), careful consideration ought to make them complementary. Here, we compare and combine estimates of ice-sheet mass balance derived from all three satellite geodetic techniques, using common spatial and temporal domains, to investigate the extent to which the approaches concur and to produce a reconciled estimate of ice-sheet mass balance.

Data and Methods

In this assessment, we use 19 years of satellite radar altimeter (RA) data, 5 years of satellite laser altimeter (LA) data, 19 years of satellite radar interferometer data, 8 years of satellite gravimetry data, 32 years of surface mass balance (SMB) model simulations, and estimates from several glacial isostatic adjustment models, to produce a reconciled estimate of ice-sheet mass balance. The satellite data sets were developed by using independent methods and, in the case of the LA, gravimeter, and SMB data sets, through contributions from numerous research groups. To enable a direct comparison, we reprocessed the geodetic data sets with use of common time intervals and common definitions of the East Antarctic, West Antarctic, Antarctic Peninsula, and Greenland ice-sheet (EAIS, WAIS, APIS, and GrIS, respectively) boundaries (16). The maximum temporal extent of the satellite data sets spans the period 1992 to 2011, and results from all geodetic techniques are available between January 2003 and December 2008. Unless stated otherwise, all results are presented with 1-sigma uncertainty estimates.

Ice-sheet surface mass balance. SMB includes solid and liquid precipitation, surface sublimation, drifting snow transport, erosion and sublimation, and meltwater formation, refreezing, retention, and runoff. Our estimates of the Antarctic Ice Sheet (AIS) and the GrIS SMB are derived from reconstructions of the RACMO2 regional atmospheric climate model (22) over the period 1979 to 2010, with horizontal resolutions of 27 (AIS) and 11 (GrIS) km. RACMO2 has a multilayer snowpack with drifting snow and snow albedo schemes (23) and has been evaluated against in situ temperature, wind, and surface-energy balance observations from weather stations (24–26) as well as satellite-derived estimates of melt extent, mass changes, and drifting snow (5, 26, 27). The spatial uncertainty of the RACMO2 mean SMB has been assessed through comparison with 310 (GrIS) and 1850 (AIS) in situ observations (28). However, temporal fluctuations in snow accumulation are poorly resolved in observation-

al data sets, so we assess the temporal uncertainty through comparison with global atmospheric reanalyses (16). We also use RACMO2 to drive a model of AIS firn densification (29) for the purpose of converting satellite LA observations into changes in ice-sheet mass.

Glacial isostatic adjustment (GIA). GIA of the solid Earth is an important contributor to the signals observed by satellite gravimetry and, to a lesser extent, satellite altimetry (30). The GIA must therefore be considered when estimating ice-sheet mass balance with either technique. In Antarctica, the use of GIA models has in practice introduced considerable uncertainty (up to 130 Gt year⁻¹) into ice-sheet mass balance estimates derived from satellite gravimetry (31–33). There are a number of contributory factors to this uncertainty, including the scarcity of constraints on the evolution of the ice sheet since the Last Glacial Maximum (LGM), limited knowledge of Earth mechanical properties, and the scarcity of near-field relative sea-level and vertical crustal motion data with which to evaluate model performance (34, 35).

Here, we consider variants of six GIA models, and we assess their impact on geodetic ice-sheet mass balance estimates. For Greenland, where the signal of GIA is relatively small and well constrained, we use the Simpson (36), ICE-5G (37), and ANU (38) models. For Antarctica, we compare the ICE-5G model (39) with two recent Antarctic GIA models: the W12a model (35, 40) and a version (IJ05_R2) of the IJ05 model (41) updated for this study (16). Both regional GIA models incorporate recently improved constraints on the ice-loading history (42–45) that suggest that the AIS was thinner at the LGM than previously thought, leading to a lowering of estimated ice-sheet mass losses since that time (40, 45). Although a consequence of this revision is a potential discrepancy between far-field sea-level records and commonly accepted Northern Hemisphere deglaciation models, both of the new regional GIA models perform well when compared with Antarctic Global Positioning System (GPS) observations (34), and we conclude that these latest solutions are best suited for estimating AIS mass balance.

Radar and laser altimetry. RA and LA provide ice-sheet mass balance estimates through measurements of ice-sheet volume change. The technique has been applied to both Greenland (46–48) and Antarctica (4, 47, 49) and is unique in spatially resolving the detailed pattern of mass imbalance, with monthly temporal sampling. RA provides the longest continuous record of all geodetic techniques (50). Altimeter measurements of elevation change are precise, because they require only modest adjustments to account for sensor drift, changes in the satellite attitude, atmospheric attenuation, and movements of Earth's surface. By far the greatest uncertainty lies in the conversion from volume to mass change. In the case of LA, this conversion has been performed by using an external model of fluctuations in the firn-

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layer thickness (29, 48, 51). In the case of RA, the conversion to mass has been performed by using a prescribed density model and by allowing for temporal fluctuations in snowfall in the uncertainty (52).

We used European Remote-Sensing (ERS-1 and ERS-2) satellite and Envisat 35-day repeat satellite RA observations to determine changes in the mass of the EAIS and WAIS between May 1992 and September 2010 (16). Time series of surface elevation change were developed at 39,375 crossing points of the satellite orbit ground tracks by using dual-cycle crossovers (49, 53). In total, 46.5 million measurements were included in this analysis, encompassing 74 and 76% of the EAIS and WAIS, respectively. The satellites were cross-calibrated by considering differences between elevation changes occurring during periods of mission overlap. Elevation data were corrected for the lag of the leading-edge tracker and for variations in dry atmospheric mass, water vapor, the ionosphere, solid Earth tides, and surface scattering (50). The IJ05_R2 model was used to correct for elevation changes associated with GIA. Mass changes were calculated by using a surface-density model with a nominal density for firn (400 kg m^{-3}) applied to all regions other than those in which changes are assumed to occur at the density of ice (900 kg m^{-3}) (52). Rates of mass change were computed in regions of interest by interpolating measurements derived at satellite-orbit crossing points and by extrapolating these results to unobserved area. To estimate the uncertainty of mass trends, we treated the estimated variability of snowfall (49) and the elevation trend variability as equivalent sources of uncertainty. This approach is used because it has not yet proved possible to separate, in the observed elevation change, annual cycles due to density fluctuations from residual variations due to signal penetration into the firn.

We used ICESat (Ice, Cloud, and Land Elevation Satellite) LA observations acquired between September 2003 and November 2008 (the period of optimal instrument calibration) to estimate changes in the mass of the AIS and GrIS (16). AIS and GrIS elevation rates were computed by four and two different groups, respectively, using methods that compare surface heights measured along repeated ground tracks. This approach provides fine along-track resolution with high precision (54). However, the ground tracks are widely separated at lower latitudes, and the elevation data are sparsely sampled in time because of the episodic nature of mission campaigns and the presence of clouds. The elevation data were corrected for the effects of GIA by using the W12a model in Antarctica and a combination of models in Greenland, and three groups corrected AIS measurements for estimates of the systematic bias between mission campaigns (32). A variety of approaches were used to isolate observations affected by clouds and to interpolate elevation rates between ground tracks. Elevation rates were adjusted for the effects of short-term

fluctuations in firn thickness by using models driven by either regional climate model predictions (29) or by remotely sensed estimates of temperature (48, 51). The error budget was calculated from uncertainties in the corrected height measurements, in the correction for change in firn thickness, and in the estimated SMB. The mass-change estimates reported here are the arithmetic average of those obtained by the different groups.

Input-output method (IOM). The IOM quantifies the difference between glacier mass gained through snowfall and lost by sublimation and meltwater runoff and the discharge of ice into the ocean. The approach has the advantage of allowing changes in SMB and ice dynamics to be examined separately at the scale of individual glacier drainage basins (5) and has been used in numerous assessments of AIS and GrIS mass balance (18, 55–57). Although earlier IOM studies used representations of SMB developed from guided interpolation of sparse ground observations (58–60), regional atmospheric climate models (5, 61) are now used because they provide sub-daily predictions at high spatial resolution that are independent of the in situ observations. When evaluated against such data, SMB model errors are found to range between 5 and 20%, depending on the basin size and location, with proportionately the largest uncertainties occurring in regions of extreme (low or high) precipitation, strong melting, or where the model resolution is too coarse. Quantifying ice-stream discharge requires measurements of ice velocity and thickness at the grounding line. Ice-sheet velocity snapshots have been widely measured by using interferometric synthetic aperture radar (InSAR) with high (<3%) accuracy (62, 63) and relatively low (annual or longer) frequency. The thickness of many ice streams has been directly measured by using airborne radar with high (~10 m) accuracy (64). Nonetheless, there are many ice-sheet outlet glaciers for which such data do not exist; in these regions, less accurate methods are used to calculate thickness with uncertainties in the range of 80 to 120 m (18, 55). Lastly, where thinning rates are large, the temporal evolution of ice thickness should be accounted for (65).

We used the IOM to determine mass changes of AIS and GrIS drainage basins between January 1992 and June 2010 (16). These results were derived according to the method of (57) and updated to include more recent data sets. SMB estimates for Greenland as reported in (66) were extended to the end of 2010. For Antarctica, the SMB estimates of (61) were used, with an updated uncertainty estimate (28). Ice discharge rates were updated by using new ice-thickness measurements in the Bellingshausen Sea sector, Wilkes Land, and the Amundsen Sea sector; at the grounding line of Filchner Ice Shelf; and at Byrd and Lambert glaciers. Direct measurements of ice thickness are now available for nearly all WAIS ice streams. The IOM inventory, including measured and derived thick-

nesses, now encompasses 64, 79, 96, and 93% of the APIS, EAIS, WAIS, and GrIS, respectively; the remainder is assumed to have no loss due to ice dynamics.

Gravimetry. The Gravity Recovery and Climate Experiment (GRACE) satellite mission has allowed fluctuations in ice-sheet mass to be estimated through measurement of their changing gravitational attraction (32, 67–69). Advantages of the GRACE method are that it provides regional averages without the need for interpolation, measures the effect of mass fluctuations directly, and permits monthly temporal sampling. However, a key challenge is to discriminate fluctuations in ice-sheet mass from changes in the underlying crust and mantle. This is achieved by using models of GIA, which, in the case of the AIS, has led to significant adjustments (70). The spatial resolution of GRACE observations derived from global spherical harmonic solutions of about 300 km in the polar regions (71) is coarse in comparison to that of other geodetic techniques. Hence, a further complicating factor is that signals may leak into regional GRACE solutions as a consequence of remote geophysical processes. In circumstances where spatial relationships between geophysical mass fluxes can be adequately characterized, application of the mass concentration unit (mascon) method (68, 72, 73) introduces a capacity to study changes at smaller scales.

Data from the GRACE satellite mission were used to estimate changes in the mass of the AIS and GrIS between January 2003 and December 2010 (16). Analysis methods varied between the six groups who contributed these observations; some used the mascon approach, whereas others used spatial-averaging kernels. The GRACE data were corrected for the effects of GIA by using the Simpson, ANU, and ICE-5G models in Greenland and the W12a, IJ05_R2, and ICE-5G models in Antarctica. Although we only include results using the W12a and IJ05_R2 models in our reconciled estimates for Antarctica, we also provide separate ice-sheet mass balance estimates determined by using the ICE-5G GIA model solution (16) to allow comparison with previously published estimates; its use leads to more-negative estimates of EAIS mass balance. Each group made its own decisions on processing the GRACE data, including how to combine results by using different GIA models, handle contamination from external sources, compute uncertainties, and compute regional mass trends and time series. Ice-sheet mass time series and trends from all groups were then averaged to obtain the individual GRACE results reported here. For all regions, the mass trends contributed by the individual groups agree with the combined GRACE trend to within the estimated uncertainties.

Results and Discussion

We investigated the extent to which the independent geodetic techniques record similar fluctuations in ice-sheet mass. First, we considered mass

changes within 52 AIS glacier drainage basins as determined by the techniques of satellite RA and IOM (Fig. 1), which are well suited to this task (14, 74). In each case, the 19-year average rate of mass loss from RA was compared with values (55) developed by using the IOM over a similar period. The average difference between the estimates of basin mass imbalance was $1.4 \pm 3.8 \text{ Gt year}^{-1}$, and there is agreement within 1- and 2-sigma uncertainty estimates in 42 and 49 of the 52 basins, respectively. Next, we computed the mass change as determined by satellite RA within areas of the

EAIS and WAIS that were beyond the scope of the IOM survey (55) to assess the extent to which the two methods are complementary (Fig. 1). These two areas, which typically fall between glacier drainage basins of the IOM survey, have small imbalances (4.5 ± 6.0 and $1.4 \pm 1.7 \text{ Gt year}^{-1}$, for the EAIS and WAIS respectively), implying that the region surveyed by the IOM is sufficient to capture the vast majority of the present EAIS and WAIS mass imbalance. Furthermore, the IOM technique is able to resolve important mass changes in regions that are beyond the effective

resolution of the RA survey, such as the APIS (Fig. 1).

As a second example, we investigated the extent to which independent geodetic techniques are able to detect fluctuations in SMB. For this exercise, we considered an exceptional snowfall event in East Antarctica during the first half of 2009 (Fig. 2). A snowfall anomaly that can be identified in CloudSat precipitation data (75) here is clearly apparent within the RACMO2 (and, hence, IOM), RA, and GRACE data sets, which record the firm thickness and mass, volume, and

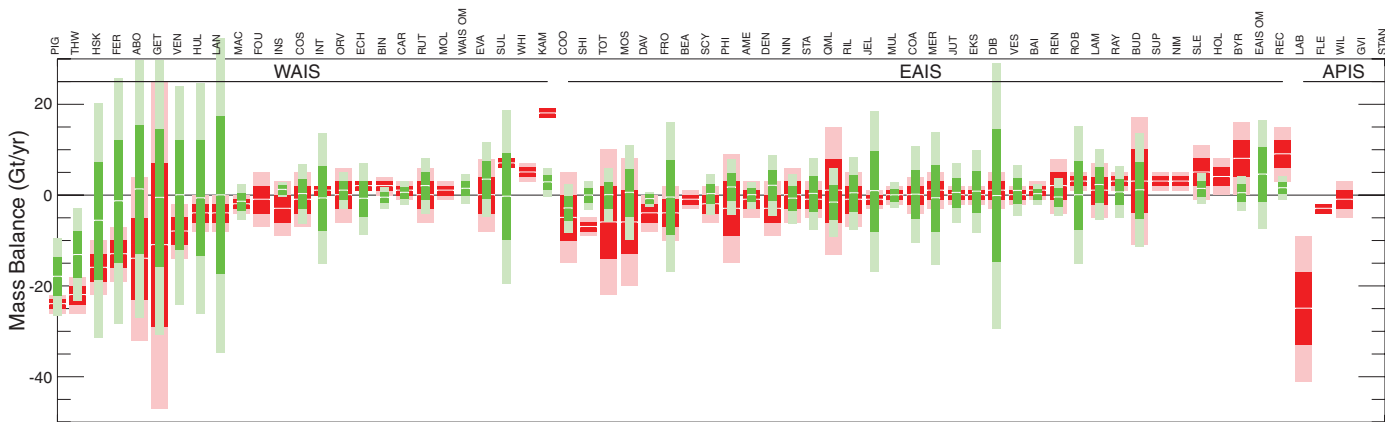


Fig. 1. Comparison of ice sheet mass balance estimates derived from satellite RA (green) and the IOM (red) over the period 1992 to 2011, with 1-sigma and 2-sigma error bars in dark and light shading, respectively, and mean values are shown in white. The comparison is performed for

52 Antarctic drainage basins (55) and the dislocated regions of East and West Antarctica that are omitted from the IOM survey (EAIS_OM and WAIS_OM, respectively). Basin locations are illustrated in the supplementary materials.

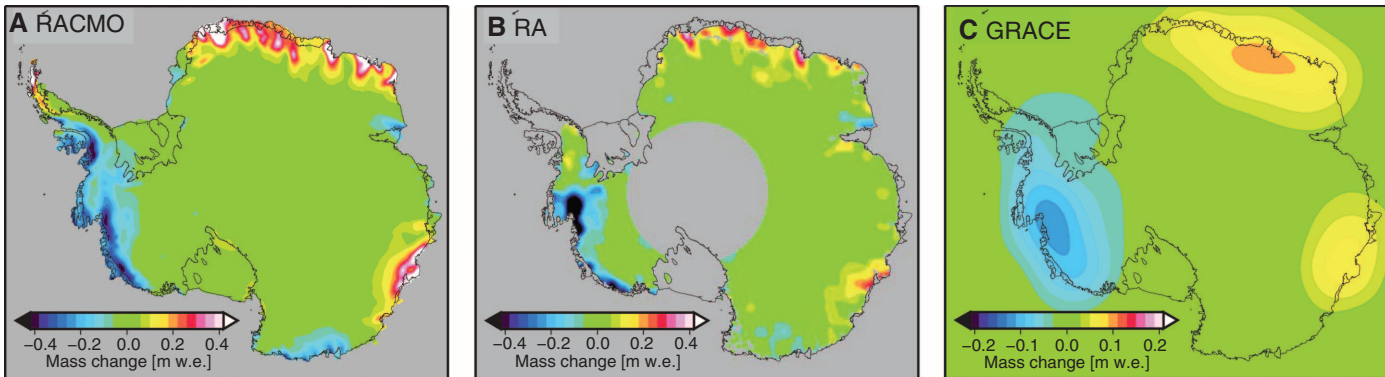
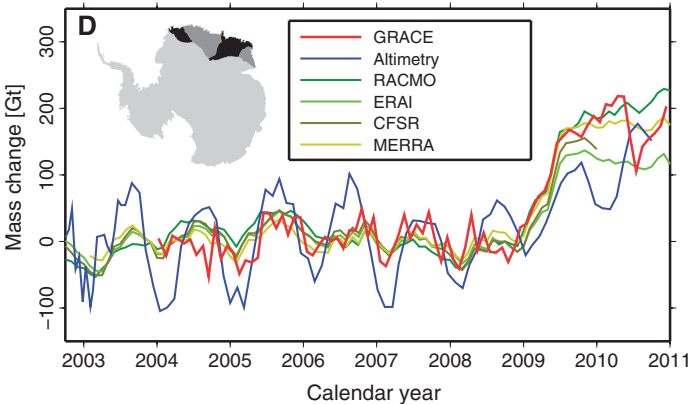


Fig. 2. Estimated anomalies in cumulative ice-sheet firm mass (A), and mass (B and C), derived from the RACMO regional climate model, satellite RA, and GRACE satellite gravimetry, respectively, over a period of anomalously high snowfall in East Antarctica. Anomalies were computed over the period July 2009 to July 2010 relative to July 2008 to July 2009. Before that, linear trends, as fitted to the 2003 to 2008 interval, were removed. The time evolution of the event, as resolved by these data sets and three additional climate models [ERA-Interim (ERA-Interim), CFSR, and MERRA], is also illustrated (D) as the average anomaly over four drainage basins of Dronning Maud Land in East Antarctica (shaded areas in inset map). Although there are SMB fluctuations elsewhere during the same time interval, the pattern of mass loss in West Antarctica is primarily associated with longer-term ice-dynamical imbalance. Relatively large annual cycles are present within some RA time series, but they do not obscure either short- or long-lived events. m w.e., meters water equivalent.



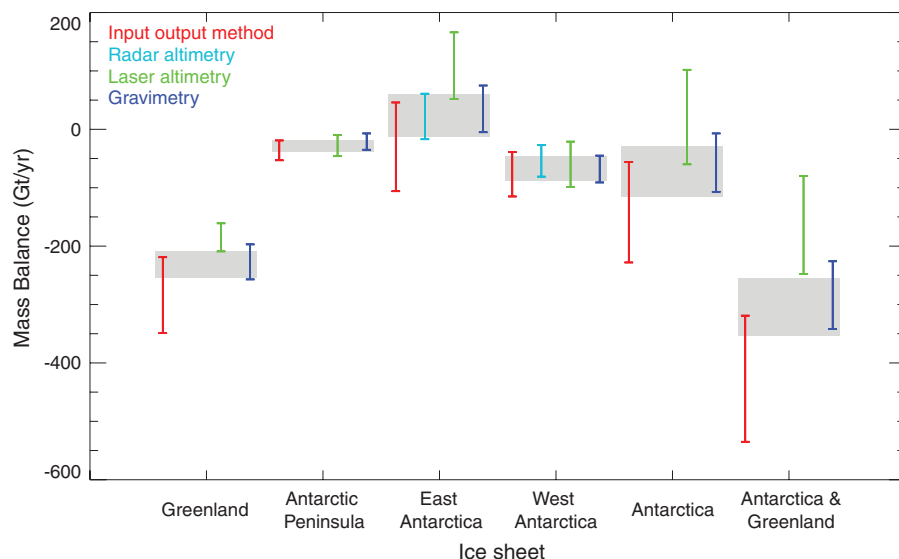


Fig. 3. Intercomparison of mass balance estimates of the GrIS, APIS, EAIS, WAIS, AIS, and the AIS plus GrIS, derived from the four independent geodetic techniques of RA (cyan), IOM (red), LA (green), and gravimetry (blue) over the period 2003 to 2008. Also shown is the reconciled result (gray).

mass fluctuations, respectively. The accumulation event affects the coastal region of the ice sheet, and, although the spatial pattern is best defined by the RACMO2 and altimeter data sets, it is also apparent in the coarser-resolution GRACE data set. Overall, around 200 Gt of additional snow mass was deposited in Dronning Maud Land during this event, equivalent to the mean annual snow accumulation in this sector of Antarctica. In addition to the snowfall anomaly, the RA and GRACE data sets also include ice-dynamical mass changes that fluctuate over the survey period, such as the accelerated mass losses in the Amundsen Sea sector.

In pursuit of a comprehensive methodological intercomparison, we computed changes in the mass of each ice-sheet region between October 2003 and December 2008, the period when all four satellite geodetic techniques were operating optimally (Fig. 3 and table S2). During this 5-year period, which is short relative to the full extent of the geodetic record and in comparison to fluctuations in SMB, the arithmetic means of ice-sheet mass imbalance estimates derived from the available geodetic techniques were -72 ± 43 and -232 ± 23 Gt year⁻¹ for the AIS and the GrIS, respectively. The technique-specific estimates agree with these mean values to within their respective uncertainties in all four ice-sheet regions and for the AIS as a whole. The only exception is the LA estimate of the combined AIS and GrIS mass imbalance, which is, at 140 ± 133 Gt year⁻¹, more positive than the mean value and only marginally beyond the 1-sigma uncertainty range of the respective values. Although the uncertainties of any one particular method are sometimes large, the combination of methods considerably improves the certainty of ice-sheet mass balance estimates.

To produce a reconciled ice-sheet mass balance estimate, we computed the average rate of mass change derived from each of the geodetic techniques within the various regions of interest and over the time periods for which geodetic mass rates were derived (Fig. 4). According to these data, ice-sheet mass balance varies cyclically and by large amounts over intermediate (2- to 4-year) time periods. For example, during the period from 1992 to 2011, the WAIS mass balance fluctuated around a mean value in the range from -50 to -100 Gt year⁻¹, but there have been episodes of considerably larger growth and loss over shorter intervals. Similar variability is apparent in other sectors of the AIS and the GrIS. We next calculated the linear average of the individual estimates of mass balance values to arrive at reconciled values and integrated these data to form a time series of cumulative mass change within each of the four ice-sheet regions (Fig. 5). Although there are obvious dependencies between the mass balance estimates produced by using each of the geodetic techniques, including, for example, the SMB data sets that are common to the IOM and LA processing, the GIA data sets that are common to the gravimetry

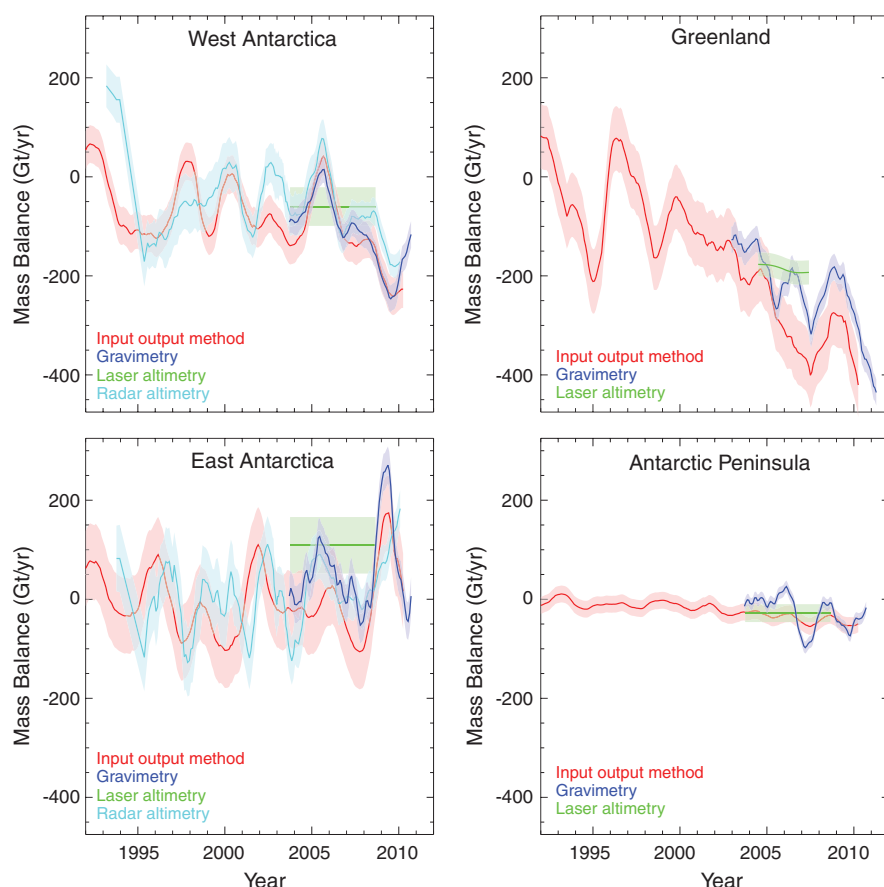


Fig. 4. Rate of mass change of the four main ice-sheet regions, as derived from the four techniques of satellite RA (cyan), IOM (red), LA (green), and gravimetry (blue), with uncertainty ranges (light shading). Rates of mass balance derived from ICESat LA data were computed as constant and time-varying trends in Antarctica and Greenland, respectively. The gravimetry and RA mass trends were computed after applying a 13-month moving average to the relative mass time series. Where temporal variations are resolved, there is often consistency in the interannual variability as determined by the independent data sets.

and altimetry processing, and the orbital corrections that are common to the LA and RA systems, these dependencies are in practice difficult to characterize. For the purpose of calculating the reconciled ice-sheet mass balance estimate, we considered IOM, gravimetry, and altimetry to be independent geodetic techniques. On the basis of this assumption, we compute the standard error of the uncertainty estimates from independent techniques as a measure of their collective uncertainty. Over the course of our 19-year survey, the average rates of mass balance of the AIS and the GrIS were -71 ± 53 and -152 ± 49 Gt year⁻¹, respectively (Table 1). For completeness, we also compute cumulative mass trends by using the data from each individual geodetic technique (fig. S1).

We also computed ice-sheet mass trends over shorter intervals to examine their variability (Table 1). These estimates, along with our integrated time series (Fig. 5), confirm several known signals of mass imbalance, including increasing mass losses from the WAIS (55, 74–77), the APIS (73, 78–80), and the GrIS (5, 81, 82). Although rates of mass loss from the GrIS were modest during the 1990s, they have increased sharply since then because of episodes of glacier acceleration (18, 83) and decreasing SMB (5, 66). GrIS glacier acceleration is, however, neither uniform nor progressive (65, 84, 85), and the large mass losses in 2010 were in fact driven by anomalously low snow accumulation and high runoff (86). The WAIS has lost mass throughout the entire survey period, and our reconciled data set shows

that the rate of mass loss has increased significantly over time (Table 1). The pattern of WAIS imbalance is dominated by mass losses (Amundsen Sea sector) and gains (Kamb Ice Stream) of dynamical origin. Although close to balance during the 1990s, there have been significant mass losses from the APIS since then because of glacier acceleration in the wake of ice-shelf collapse (87, 88) and calving-front retreat (77, 89). The APIS now accounts for around 25% of all mass losses from Antarctic regions that are in a state of negative mass balance, despite occupying just 4% of the continental area. In contrast, the EAIS, which occupies over 75% of Antarctica, was in approximate balance throughout the 1990s. Although the EAIS has experienced mass gains during the final years of our survey (Table 1 and Fig. 5), our reconciled data set is too short to determine whether they were caused by natural fluctuations that are a common feature of Antarctic ice-core records (90) or long-term increases in precipitation that are a common feature of global and regional climate model projections (91–93). Both satellite altimeter data sets highlight the lower reaches of the Cook and Totten Glaciers as regions of ice dynamical mass loss (15, 77), but neither signal is large in comparison with the wider EAIS mass trend. Overall, snowfall-driven mass gains in East Antarctica, notably the anomalous event in Dronning Maud Land during 2009 (Fig. 2), have reduced the rate at which Antarctic ice losses have increased over time, but the EAIS record is too short to determine whether this is a long-term trend.

Our reconciliation exercise has highlighted several other issues. Assessments of GrIS mass balance require more careful consideration than was possible here, because the surrounding mountain glaciers and ice caps are included in some, but not all, of our geodetic surveys and because the ice-sheet domains varied in area by 2%. One estimate has put their contribution at ~ 20 Gt year⁻¹ (94), a value that falls between two we have derived ourselves from ICESat data (10 and 40 Gt year⁻¹). For the EAIS, our mass change estimates exhibit an unsatisfactory spread, with results from the IOM and LA techniques falling consistently lower and higher than the mean value we have derived (table S2). Although the average signal of EAIS imbalance is relatively small, such a large divergence is a matter of concern; improvements of the ancillary data sets that support satellite observations would be of considerable benefit in this region. Lastly, the spatial sampling of mass fluctuations at the APIS is at present inadequate, particularly considering that it provides a significant component of the overall AIS imbalance. Improvements in the spatial and temporal density of satellite observations of this region are needed.

Fig. 5. Cumulative changes in the mass of (left axis) the EAIS, WAIS, and APIS (**top**) and GrIS and AIS and the combined change of the AIS and GrIS (**bottom**), determined from a reconciliation of measurements acquired by satellite RA, the IOM, satellite gravimetry, and satellite LA. Also shown is the equivalent global sea-level contribution (right axis), calculated assuming that 360 Gt of ice corresponds to 1 mm of sea-level rise. Temporal variations in the availability of the various satellite data sets (Fig. 4) means that the reconciled mass balance is weighted toward different techniques during certain periods.

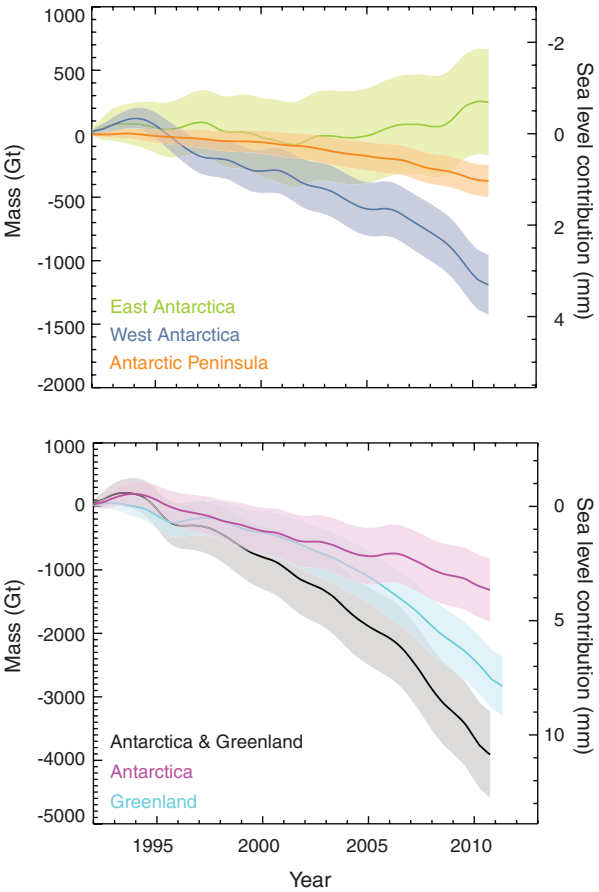


Table 1. Reconciled ice-sheet mass balance estimates determined during various epochs, inclusive of all data present during the given dates. The period 1993 to 2003 was used in an earlier assessment (2).

Region	1992–2011 (Gt/year)	1992–2000 (Gt/year)	1993–2003 (Gt/year)	2000–2011 (Gt/year)	2005–2010 (Gt/year)
GrIS	-142 ± 49	-51 ± 65	-83 ± 63	-211 ± 37	-263 ± 30
APIS	-20 ± 14	-8 ± 17	-12 ± 17	-29 ± 12	-36 ± 10
EAIS	14 ± 43	-2 ± 54	-9 ± 50	26 ± 36	58 ± 31
WAIS	-65 ± 26	-38 ± 32	-49 ± 31	-85 ± 22	-102 ± 18
AIS	-71 ± 53	-48 ± 65	-71 ± 61	-87 ± 43	-81 ± 37
GrIS + AIS	-213 ± 72	-100 ± 92	-153 ± 88	-298 ± 58	-344 ± 48

Conclusions

We estimate that, between 1992 and 2011, the Antarctic and Greenland ice sheets lost 1350 ± 1010 and 2700 ± 930 Gt of ice, respectively, equivalent to an increase in global mean sea level

of 11.2 ± 3.8 mm. The greater certainty and comprehensive nature of this estimate is made possible through our combination of observations derived from a range of geodetic techniques, each of which has different strengths and weaknesses. We have quantified and characterized the ice-sheet imbalance associated with glacier dynamics (the APIS and WAIS), SMB (the EAIS), and a mixture of the two processes (the GrIS). We have also identified the geographical regions where improved data sets are required; the APIS and the EAIS would benefit from measurements with greater spatial sampling and longer temporal sampling, respectively. Although measurements from new and future satellite missions, such as CryoSat-2, may offer new data with which to tackle the former of these challenges, improvements to ancillary data sets are also required. We have shown that assessments of mass imbalance based on short geodetic records should be treated with care, because fluctuations in SMB can be large over short time periods. Lastly, our assessment demonstrates that geodetic assessments of ice-sheet mass balance should consider both the spatial and temporal limitations of the particular data sets upon which they are based and their value in relation to findings based on other independent approaches.

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Supplementary Materials

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The Imprint of the Extragalactic Background Light in the Gamma-Ray Spectra of Blazars

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The light emitted by stars and accreting compact objects through the history of the universe is encoded in the intensity of the extragalactic background light (EBL). Knowledge of the EBL is important to understand the nature of star formation and galaxy evolution, but direct measurements of the EBL are limited by galactic and other foreground emissions. Here, we report an absorption feature seen in the combined spectra of a sample of gamma-ray blazars out to a redshift of $z \sim 1.6$. This feature is caused by attenuation of gamma rays by the EBL at optical to ultraviolet frequencies and allowed us to measure the EBL flux density in this frequency band.

The bulk of the intergalactic gas in the universe must have been reionized between the epoch of cosmic recombination, when the universe was only 300,000 years old ($z \sim 1100$), and 1 billion years later ($z \sim 6$), as indicated observationally by the spectra of distant quasistellar objects (1). However, the sources, modes, and nature of this cosmic reionization are largely unknown because most of this redshift range has yet to be explored. Photoionization by ultraviolet (UV) radiation, produced by the first stars and galaxies of the universe, represents the primary suspect for the ionizing process (2, 3). Direct detection of the UV radiation fields is thus of fundamental importance, but at present is extremely difficult (3).

An indirect but powerful means of probing the diffuse radiation fields is through γ - γ absorption of high-energy gamma rays (4–6). In this process, a gamma-ray photon of energy E_γ and an extragalactic background light (EBL) photon of energy E_{EBL} annihilate and create an electron-positron pair. This process occurs for head-on collisions when, for example, $E_\gamma \times E_{\text{EBL}} \geq 2(m_e c^2)^2$, where $m_e c^2$ is the rest mass energy of the elec-

tron. This introduces an attenuation in the spectra of gamma-ray sources above a critical gamma-ray energy of $E_{\text{crit}}(z) \approx 170(1+z)^{-2.38}$ GeV (7, 8).

The detection of the gamma-ray horizon (i.e., the point beyond which the emission of gamma-ray sources is strongly attenuated) is one of the primary scientific drivers of the Fermi Gamma-Ray Space Telescope (9–11). Several attempts have been made in the past, but none detected the long-sought EBL attenuation (12–14). So far, limits on the EBL density have been inferred from the absence of absorption features in the spectra of individual blazars (13, 15), distant galaxies with bright gamma-ray emission powered by matter accreting onto central, massive black holes. Although this feature is indeed difficult to constrain for a single source, we show that it is detected collectively in the gamma-ray spectra of a sample of blazars as a cutoff that changes amplitude and energy with redshift. We searched for an attenuation of the spectra of blazars in the 1 to 500 GeV band using the first 46 months of observations of the Large Area Telescope (LAT) on board the Fermi satellite. At these energies, gamma rays

are absorbed by EBL photons in the optical to UV range. Thanks to the large energy and redshift coverage, Fermi-LAT measures the intrinsic (i.e., unabsorbed) spectrum up to ~ 100 GeV for any blazar at $z < 0.2$ and up to ~ 15 GeV for any redshift.

The LAT has detected >1000 blazars to date (16). We restricted our search to a subset of 150 blazars of the BL Lacertae (BL Lac) type that are significantly detected above 3 GeV because of the expected lack of intrinsic absorption (17). The sample covers a redshift range of 0.03 to 1.6 (18, 19). The critical energy is therefore always ≥ 25 GeV, which means that the spectrum measured below this energy is unabsorbed and a true representation of the intrinsic spectrum of the source. We thus determined the intrinsic source spectrum relying on data between 1 GeV and the critical energy E_{crit} and extrapolated it to higher energies. By combining all the spectra, we were able to determine the average deviation, above the critical energy, of the measured spectra from the intrinsic ones, which ultimately provides a measurement of the optical depth $\tau_{\gamma\gamma}$.

The analysis was performed using the Fermi Science Tools (20). We determined the spectral parameters of each blazar by maximizing the likelihood of a given source model. The model comprised the Galactic and isotropic diffuse components and all sources in the second Fermi LAT catalog (21) within a region of interest (ROI) of 15° radius. We modeled the spectra of the sources in our sample as parabolic in the logarithmic space of energy and flux [see equation 2 in (21) for a definition]. Their spectra were modified by a term $e^{-\tau_{\gamma\gamma}(E,z)}$ that describes the absorption of gamma-ray photons on the EBL. In the above, we defined $\tau_{\gamma\gamma}(E,z) = b \times \tau_{\gamma\gamma}^{\text{model}}(E,z)$, where the $\tau_{\gamma\gamma}^{\text{model}}(E,z)$ is the optical depth predicted by EBL models (7, 22–25) and b is a scaling variable, left free in the likelihood maximization. In particular, this allowed us to assess the likelihood of two important scenarios: (i) there is no EBL attenuation ($b = 0$), or (ii) the model prediction is correct ($b = 1$).

We combined the data from all the ROIs in a global fit that determined the common parameter b for a given EBL model (see table S1). All those models with a minimal EBL density based on (or compatible with) resolved galaxy counts (2, 7, 24–27) were found to be acceptable descriptions of the Fermi data (i.e., are consistent with $b = 1$ within $\approx 25\%$) (see also Fig. 1), yielding a significance of the absorption feature of up to ~ 6 SD. Models that predict a larger intensity of the EBL, particularly in the UV (22, 23), would produce a stronger-than-observed attenuation feature and are therefore incompatible with the Fermi observations. Our measurement points to a minimal level of the optical-UV EBL up to redshift $z \approx 1.6$, which combined with the upper limits (15, 28, 29) derived at lower redshift (using observations of blazars at TeV energies) on the near-infrared EBL highlights the conclusion that most of the EBL intensity can be explained by the measured galaxy emission.

Our measurement relies on the accuracy of the extrapolation of the intrinsic spectra of the

sources above the critical energy (30). This in turn depends on a precise description of the gamma-ray spectra by our source parametrization. To verify that this is the case and to exclude the possibility that the detected absorption feature is intrinsic to the gamma-ray sources (17), we performed the analysis in three independent redshift intervals ($z < 0.2$, $0.2 \leq z < 0.5$, and $0.5 \leq z < 1.6$). The deviations from the intrinsic spectra in the three redshift intervals are displayed in Fig. 2. In the local universe ($z < 0.2$), EBL absorption is negligible in most of the Fermi-LAT energy

band ($E_{\text{crit}} \geq 120$ GeV). The lowest redshift interval therefore reveals directly the intrinsic spectra of the sources and shows that our spectral parametrization is accurate (18). The absorption feature is clearly visible above the critical energy in the higher redshift bins. Its amplitude and modulation in energy evolve with redshift as expected for EBL absorption. In principle, the observed attenuation could be due to a spectral cutoff that is intrinsic to the gamma-ray sources. The absence of a cutoff in the spectra of sources with $z < 0.2$ would require that the properties of BL Lacs change

with redshift or luminosity. It remains an issue of debate whether such evolution exists (31–34). However, in case it were present, the intrinsic cutoff would be expected to evolve differently with redshift than we observe. To illustrate this effect, we fitted the blazar sample assuming that all the sources have an exponential cutoff at an energy E_0 . From source to source, the observed cutoff energy changes because of the source redshift and because we assumed that blazars as a population are distributed in a sequence such as that proposed in (31–34). E_0 was fitted to the data globally like b above. As

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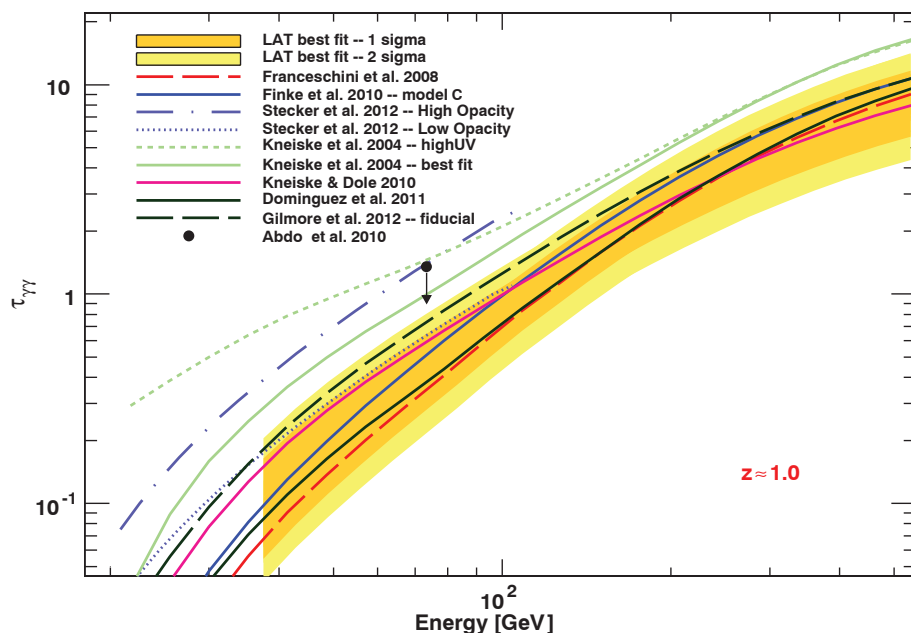
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Fig. 1. Measurement, at the 68 and 95% confidence levels (including systematic uncertainties added in quadrature), of the opacity $\tau_{\gamma\gamma}$ from the best fits to the Fermi data compared with predictions of EBL models. The plot shows the measurement at $z \approx 1$, which is the average redshift of the most constraining redshift interval (i.e., $0.5 \leq z < 1.6$). The Fermi-LAT measurement was derived combining the limits on the best-fit EBL models. The downward arrow represents the 95% upper limit on the opacity at $z = 1.05$ derived in (13). For clarity, this figure shows only a selection of the models we tested; the full list is reported in table S1. The EBL models of (49), which are not defined for $E \geq 250/(1+z)$ GeV and thus could not be used, are reported here for completeness.



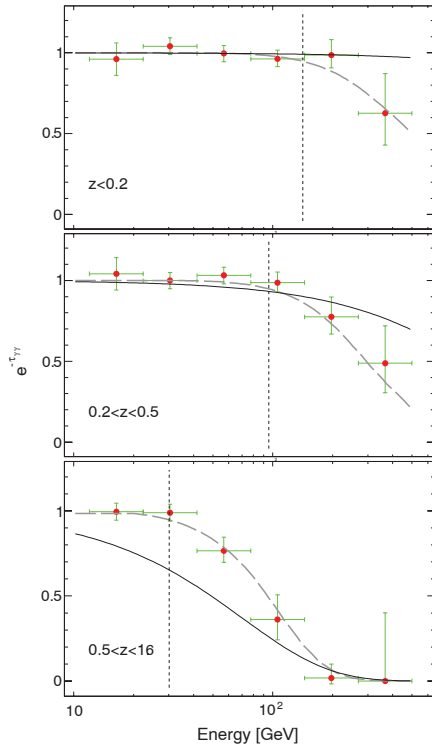


Fig. 2. Absorption feature present in the spectra of BL Lac objects as a function of increasing redshift (data points, from top to bottom). The dashed curves show the attenuation expected for the sample of sources by averaging, in each redshift and energy bin, the opacities of the sample [the model of (7) was used] and multiplying this average by the best-fit scaling parameter b obtained independently in each redshift interval. The vertical line shows the critical energy E_{crit} below which $\leq 5\%$ of the source photons are absorbed by the EBL. The thin solid curve represents the best-fit model, assuming that all the sources have an intrinsic exponential cutoff and that blazars follow the blazar sequence model of (32, 33).

apparent from Fig. 2, it appears difficult to reconcile the observed feature with an intrinsic characteristic of the blazars' spectra. We therefore associate the spectral feature to the EBL absorption.

At energies ≤ 100 GeV, gamma rays observed at Earth and coming from redshift ≥ 1 interact mostly with UV photons of ≥ 5 electron volts. An UV background in excess of the light emitted by resolved galaxies can be produced locally by active galactic nuclei (AGN) or at higher redshift ($z \approx 7$ to 15) by low-metallicity massive stars (35). By comparing the results from the best-fit EBL models, we measured the UV component of the EBL to have an intensity of $3(\pm 1) \text{ nW m}^{-2} \text{ sr}^{-1}$ at $z \approx 1$. A contribution to the UV background from AGN as large as the one predicted by (36) (i.e., $\approx 10 \text{ nW m}^{-2} \text{ sr}^{-1}$) and used in the EBL model of (22) is thus excluded by our analysis at high confidence. However, the recent prediction (37) of the UV background from AGN ($\approx 2 \text{ nW m}^{-2} \text{ sr}^{-1}$) is in agreement with the Fermi measurement. Direct measurements of the extragalactic UV background are hampered by the strong dust-scattered Galactic radiation (38). The

agreement between the intensity of the UV background as measured with Fermi and that due to galaxies individually resolved by the Hubble Space Telescope (39) ($3 \pm 1 \text{ nW m}^{-2} \text{ sr}^{-1}$ versus $2.9\text{--}3.9 \text{ nW m}^{-2} \text{ sr}^{-1}$, respectively) shows that the room for any residual diffuse UV emission is small. This conclusion is reinforced by the good agreement of the Fermi measurement and the estimate of the average UV background, at $z \geq 1.7$, of 2.2 to $4.0 \text{ nW m}^{-2} \text{ sr}^{-1}$ using the proximity effect in quasar spectra (40).

Zero-metallicity population-III stars or low-metallicity population-II stars are thought to be the first stars to form in the universe and formally marked the end of the dark ages when, with their UV light, these objects started ionizing the intergalactic medium (41). These stars, whose mass might have exceeded 100 times the mass of our Sun ($M_{\odot}$), are also believed to be responsible for creating the first metals and dispersing them in the intergalactic medium (42–44). A very large contribution of population-III stars to the near-infrared EBL had already been excluded by (15). Our measurement constrains, according to (45, 46), the redshift of maximum formation of low-metallicity stars to be at $z \geq 10$ and its peak comoving star-formation rate to be lower than $0.5 M_{\odot} \text{ Mpc}^{-3} \text{ year}^{-1}$. This upper limit is already of the same order of the peak star-formation rate of 0.2 to $0.6 M_{\odot} \text{ Mpc}^{-3} \text{ year}^{-1}$ proposed by (47) and suggests that the peak star-formation rate might be much lower, as proposed by (48).

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Supplementary Materials

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Superconducting Dome in a Gate-Tuned Band Insulator

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A dome-shaped superconducting region appears in the phase diagrams of many unconventional superconductors. In doped band insulators, however, reaching optimal superconductivity by the fine-tuning of carriers has seldom been seen. We report the observation of a superconducting dome in the temperature–carrier density phase diagram of MoS₂, an archetypal band insulator. By quasi-continuous electrostatic carrier doping achieved through a combination of liquid and solid gating, we revealed a large enhancement in the transition temperature T_c occurring at optimal doping in the chemically inaccessible low-carrier density regime. This observation indicates that the superconducting dome may arise even in doped band insulators.

In many unconventional superconductors (1–3), the transition temperature T_c has a maximum as a function of external parameters such as chemical doping or pressure; in cuprate families, this so-called superconducting dome arises upon the chemical doping of the parent Mott insulator (1). In band insulators, similar behavior was observed at low carrier densities (4) where superconductivity is usually not favorable because of the low density of states (DOS)

(4, 5). Except in certain cases (4, 6), however, using chemical doping to achieve low carrier densities results in nonuniformity or phase separation. Other systems exhibiting optimal doping of the superconducting state include two-dimensional (2D) electron systems at surfaces and interfaces, whose phase diagrams may be explored by applying electric fields (7–9). In recent years, this electrostatic carrier doping has been effectively implemented by using ionic liquids to form an electrical double layer (EDL) of high capacitance (10–12). This method has produced carrier densities that span the superconducting dome in high- T_c cuprates (13, 14) and may be an effective tool to access exotic superconducting states in other materials.

We chose a typical band insulator, MoS₂, because the high mobility found in its solid-state

transistor operations (15) suggests that interesting basic physical properties may be revealed by using the EDL dielectrics (11, 16–18). To make our devices, we isolated thin flakes of MoS₂ from a bulk 2H-type single crystal (Fig. 1A) by the Scotch tape method widely used in graphene research (19, 20) and transferred them onto the surface of HfO₂ grown by atomic layer deposition on a Nb-doped SrTiO₃ substrate. We selected atomically flat thin flakes by examining their optical micrographs (21) and patterned them into a Hall bar configuration (Fig. 1B), which acts as a transistor channel (11, 16). A droplet of ionic liquid (DEME-TSFI) (21) was applied onto the surface of the thin flake covering the side gate electrode (Fig. 1C). A voltage applied between the thus formed liquid gate (LG) and the channel drives either anions or cations onto the channel surface under positive or negative bias, respectively. The ions and induced carriers ($\sim 10^{14}$ cm⁻²) right beneath form an equivalent capacitance of ~ 10 μ F/cm², large enough for inducing superconductivity at the interface (10–14). In addition, we were able to modulate the carrier density (to $\sim 10^{13}$ cm⁻²) using a high- k dielectric (HfO₂) back gate (BG), which remains effective after the freezing of the ion motion at a temperature below ~ 200 K. For carriers induced at the top surface of the MoS₂ flake by the LG, the effective BG capacitance is affected by two layers of dielectrics: HfO₂ and the bulk of MoS₂ flake. Using this double gating method, we could access a large range of carrier densities n_{2D} quasi-continuously and precisely, thereby avoiding the staging effect (22), even in the low-density

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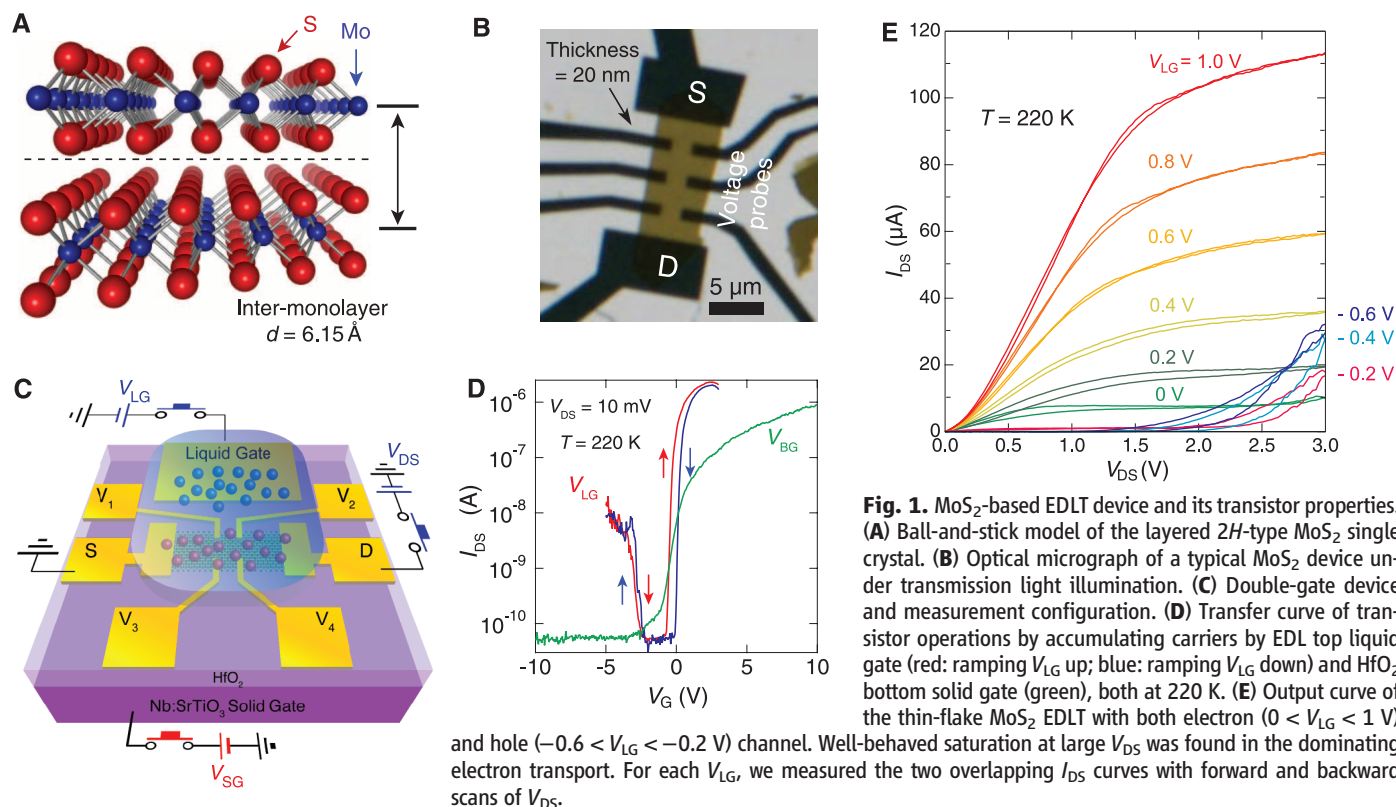


Fig. 1. MoS₂-based EDLT device and its transistor properties. (A) Ball-and-stick model of the layered 2H-type MoS₂ single crystal. (B) Optical micrograph of a typical MoS₂ device under transmission light illumination. (C) Double-gate device and measurement configuration. (D) Transfer curve of transistor operations by accumulating carriers by EDL top liquid gate (red: ramping V_{LG} up; blue: ramping V_{LG} down) and HfO₂ bottom solid gate (green), both at 220 K. (E) Output curve of the thin-flake MoS₂ EDLT with both electron ($0 < V_{LG} < 1$ V) and hole ($-0.6 < V_{LG} < -0.2$ V) channel. Well-behaved saturation at large V_{DS} was found in the dominating electron transport. For each V_{LG} , we measured the two overlapping I_{DS} curves with forward and backward scans of V_{DS} .

regime where chemical doping is plagued by nonuniformity.

Figure 1D shows the transfer curves of a typical double-gate device at 220 K with a source-drain voltage $V_{DS} = 10$ mV. For the n -channel conduction, an on/off ratio of $>10^4$ was reached for biasing with either the liquid ionic gate (V_{LG}) or the high- k back gate (V_{BG}), with a channel resistance $R_{DS} > 1$ gigohm in the off state. Compared with the BG, the LG not only had 10 times the gate efficiency (the change of channel current versus gate voltage $\frac{\Delta I_{DS}}{\Delta V_G}$), but also created an additional p-channel when a negative V_G was applied. This ambipolar transport indicates that the LG is effective in shifting the Fermi level E_F to access both the valence and conduction bands (16). An enhanced p-channel with more balanced

ambipolar transport could be found in flakes with less intrinsic electron doping (sulfur deficiency), where the barely metallic state in the p-channel was still far from reaching hole superconductivity (16). To confirm the electrostatic operation of LG, we performed a transfer curve measurement with fast gate bias cycles (fig. S1). The possibility of a chemical reaction was ruled out by repeatability and a negligible (~ 1 nA) leak current I_G , as well as a persistent off state (> 1 gigohm). The I_{DS} versus V_{DS} characteristic in the output operation (Fig. 1E) of a typical MoS₂ EDLT transistor (EDLT) corroborates the more pronounced n -channel ($0 < V_{LG} < 1$ V) than p -channel operation ($-0.6 < V_{LG} < -0.2$ V), consistent with the transfer characteristics. Compared with the n -channel operation observed in monolayer de-

vices (15), the more pronounced saturation at high V_{DS} indicates well-behaved transistor operation.

After introducing carriers onto the channel surface at 220 K with different liquid-gate biases V_{LG} , we measured the four-terminal sheet resistance R_s as a function of temperature T when the device was being cooled down to 2 K (Fig. 2A). At gate biases $V_{LG} < 1$ V, we observed a negative temperature derivative of R_s (dR_s/dT) for insulating states. The increase of dR_s/dT with V_{LG} indicates the gradual formation of degenerate carriers and enhanced mobility at low temperatures. The channel surface shows metallic transport (positive dR_s/dT) at $V_{LG} \geq 1$ V. The enhancement of metallicity continues with further increase of V_{LG} , and superconductivity emerges at $V_{LG} = 4$ V. The transition temperature T_c shows clear V_{LG}

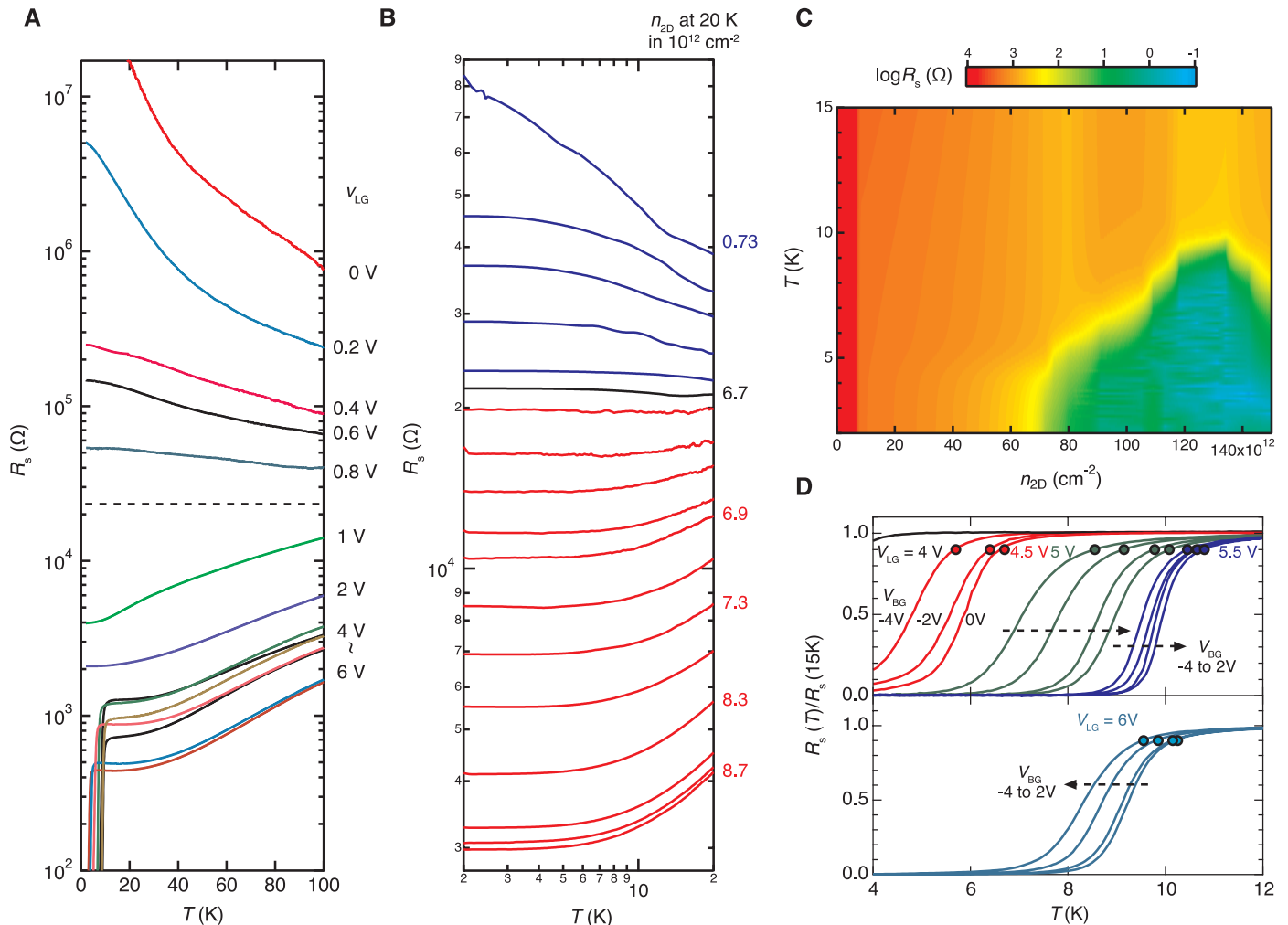


Fig. 2. Transport properties of the thin-flake MoS₂ EDLT. **(A)** Temperature dependence of the channel sheet resistance R_s at different V_{LG} gate biases ranging from 0 to 6 V (indicated on the right). **(B)** Temperature dependence of the channel sheet resistance R_s at $V_{LG} = 1$ V and different V_{BG} 's showing a metal-insulator transition at $n_{2D} = 6.7 \times 10^{12}$ cm⁻². For each V_{BG} , we marked the corresponding n_{2D} measured by the Hall effect at 20 K. **(C)** Phase diagram showing the evolution of different electronic phases as a function of carrier density n_{2D} . The phase diagram shows an insulating ($n_{2D} < 6.7 \times 10^{12}$ cm⁻²), a metallic ($6.7 \times 10^{12} < n_{2D} < 6.8 \times 10^{13}$ cm⁻²), and a dome-like superconducting phase ($n_{2D} > 6.8 \times 10^{13}$ cm⁻²), where the color cor-

responds to the logarithm of the sheet resistance R_s (Ω). **(D)** Normalized superconducting transition $R_s(T)/R_s(15K)$ as a function of temperature for different gate voltages. The T_c is marked as a circle at 90% of the total transition. Both V_{LG} and V_{BG} are varied; for a given V_{LG} , we show the evolution of the transition with increasing V_{BG} . All data corresponding to the same V_{LG} are shown with the same color. The dashed arrows indicate the order of increasing V_{BG} from -4 to 2 V in $\Delta V_{BG} = 2$ V. (Upper panel) For V_{LG} between 4 and 5.5 V, T_c increased with increasing V_{BG} ; (bottom panel) for $V_{LG} = 6$ V, T_c decreased with increasing V_{BG} because the corresponding n_{2D} had reached the peak of the superconducting dome.

dependence until V_{LG} reaches 6 V, the maximum voltage used in our experiment. Similar field-induced superconductivity was reported recently by Taniguchi *et al.* (23).

For the metallic states at $V_{LG} = 1$ V, we switched on the solid back gate to study the metal insulator transition (MIT) by a precise control of the transport with 18 different n_{2D} values ($7.3 \times 10^{11} < n_{2D} < 8.7 \times 10^{12} \text{ cm}^{-2}$) measured by the Hall effect at 20 K (Fig. 2B). A transition between the insulating (blue) and metallic (red) transport was clearly separated by a critical resistance $R_c = 21.7$ kilohm (separatrix, black line) at $n_c = 6.7 \times 10^{12} \text{ cm}^{-2}$, close to the quantum resistance h/e^2 , consistent with MITs found in other 2D systems (24). A Hall mobility of $\mu_H \sim 240 \text{ cm}^2/\text{Vs}$ at $n_{2D} = 8.7 \times 10^{12} \text{ cm}^{-2}$ is comparable to the bulk value (25), supporting our choice of (~ 20 nm thick) flakes instead of monolayers, where ripples might lower the mobility (26, 27). After the formation of metallic states, increasing the gate bias also creates a growing perpendicular surface electric field E_s , inducing spin-orbit interaction, which corroborates the 2D nature of MoS₂ interface (21, 28). Varying both V_{LG} and V_{BG} , we quasi-continuously mapped $\log R_s$ in the n_{2D} and T plane (Fig. 2C). This enables a detailed study of the full superconducting phase induced by the field effect in a pristine compound, avoiding a nonuniform dopant distribution or phase separation. At several fixed values of V_{LG} , we manipulated T_c by varying V_{BG} (Fig. 2D).

Figure 3A shows a superconducting phase diagram of MoS₂ as a function of n_{2D} measured by

Hall effect at 20 K. As estimated from the Thomas-Fermi screening length for $n_{2D} \sim 10^{14} \text{ cm}^{-2}$, we assumed that the carriers are accumulated in a half of one unit cell, $6.15 \text{ \AA}$ (a monolayer). Notably, this value of n_{2D} can be regarded as the upper limit to the estimate of n_{2D} when we unify our phase diagram with that of alkali metal-intercalated 2H-MoS₂ in Fig. 3A (29, 30). The superconductivity sharply appears at $n_{2D} = 6.8 \times 10^{13} \text{ cm}^{-2}$, then saturates after reaching a maximum $T_c = 10.8 \text{ K}$ at $n_{2D} = 1.2 \times 10^{14} \text{ cm}^{-2}$, followed by a decrease in T_c at larger n_{2D} ; this results in a dome-like superconducting state. We defined T_c as the temperature at which R_s reaches 90% of its normal state value. At the onset of the superconducting phase, the critical behavior near the quantum critical point could be well fitted with $T \propto (n_{2D} - n_0)^{z\nu}$, where $z\nu = 0.6$ (fig. S4), in a manner similar to that for LaAlO₃/SrTiO₃ interfaces (7). This is also consistent with $z\nu = 0.5 \sim 0.6$ found in boron-doped diamond, where a comparable T_c was observed (31).

In the combined phase diagram, which includes the chemically doped MoS₂ (29, 30), the field-induced phase showed enhanced T_c ($\sim 40\%$ higher than the maximum T_c found in Cs_xMoS₂) at a much lower n_{2D} (Fig. 3A). The maximum T_c is also well above that of NbSe₂ ($\sim 7 \text{ K}$), which was thought to be the highest T_c in transition-metal dichalcogenides. That the field-induced phase appears with an enhanced T_c at a much lower n_{2D} with respect to the alkali metal-doped compounds confirms the effectiveness of searching for different regions of carrier concentrations in doped semiconductors. It appears that the de-

crease in T_c connects smoothly to the region of bulk superconductivity for alkali metal-doped MoS₂ compounds.

To understand these superconducting features found in MoS₂ EDLT, we calculated its electronic structure for both monolayer and bulk (21). Figure 3B shows the DOS spectrum for the electron-doped monolayer MoS₂, which mimics the gate-induced charge accumulation layer at large carrier densities of $\sim 10^{14} \text{ cm}^{-2}$. The conduction band edge consists predominantly of the d_{z^2} orbital of Mo. Reflecting the 2D nature of the monolayer, the DOS is nearly flat in the region of $6.8 \times 10^{13} < n_{2D} < 1.2 \times 10^{14} \text{ cm}^{-2}$, where superconductivity sharply sets in and reaches the maximum T_c , corresponding to an E_F shift of 0.25 eV from the band edge (21). The DOS continues to increase with n_{2D} owing to the contribution from additional $d_{x^2-y^2}$ and d_{xy} orbitals, indicating that the decrease in T_c above $1.2 \times 10^{14} \text{ cm}^{-2}$ is not simply controlled by the change in the DOS.

Such a decrease in T_c with increasing carrier density was first recognized in Na-doped WO₃ and was attributed to phonon softening from a structural transition observed in the vicinity of the superconducting phase (32). However, the phonon softening scenario should not apply to MoS₂ because its structural transition occurs at an order of magnitude higher n_{2D} (33). A similar superconducting dome with a quantum critical point has been recently observed in electric-field-controlled interface superconductivity such as in LaAlO₃/SrTiO₃ (7) and KTaO₃ (12). Thus, it is possible that a more universal, non-material-

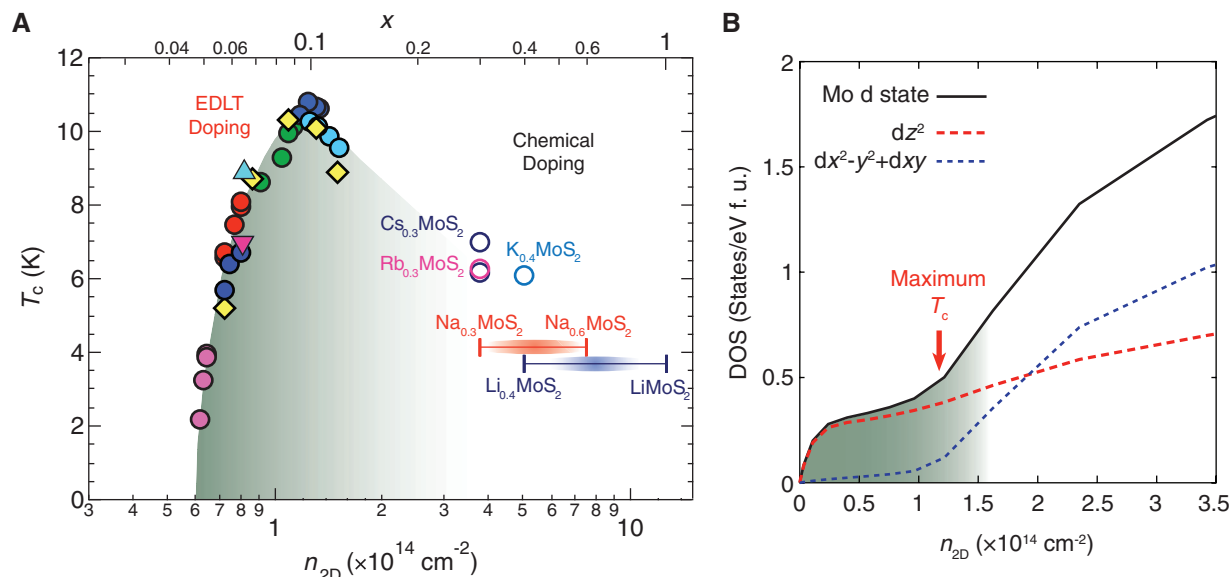


Fig. 3. Phase diagram and band calculation of electron-doped MoS₂. **(A)** Unified phase diagram of superconductivity of both electrostatically and chemically doped MoS₂ as a function of doping concentration x (upper horizontal axis) and carrier density n_{2D} (bottom horizontal axis). The field-induced superconducting data were from four different samples, each marked with a differently shaped filled symbol. Filled circles of the same color correspond to the superconducting states at a fixed V_{LG} but different V_{BG} 's.

Open circles show the T_c of MoS₂ chemically intercalated with different alkali metal dopants. Solid bars denote the range of doping showing the same T_c . The structure of all intercalated compounds is 2H-type within the indicated carrier density region. **(B)** Calculated DOS of MoS₂. The field-induced carriers mainly occupy the 4d state of Mo, covering the n_{2D} region indicated by the shaded green area. The red arrow locates the n_{2D} where the maximum T_c was observed.

specific model exists for the superconducting phase diagram in doped band insulators at dilute carrier densities.

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Formation of Regular Satellites from Ancient Massive Rings in the Solar System

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When a planetary tidal disk—like Saturn’s rings—spreads beyond the Roche radius (inside which planetary tides prevent aggregation), satellites form and migrate away. Here, we show that most regular satellites in the solar system probably formed in this way. According to our analytical model, when the spreading is slow, a retinue of satellites appear with masses increasing with distance to the Roche radius, in excellent agreement with Saturn’s, Uranus’, and Neptune’s satellite systems. This suggests that Uranus and Neptune used to have massive rings that disappeared to give birth to most of their regular satellites. When the spreading is fast, only one large satellite forms, as was the case for Pluto and Earth. This conceptually bridges the gap between terrestrial and giant planet systems.

Satellites are generally thought to form concurrently with a giant planet, in a large circumplanetary gaseous disk where there is inflow of solids. Two competing models exist in the literature (1–4), in which solids aggregate to form satellites that can migrate in the gas (and possibly be lost) before the gas dissipates. These models have their pros and cons, but none can explain the surprising orbital architecture of Saturn’s, Uranus’, and Neptune’s satellite systems, where the smallest bodies accumulate at a distance from the planet that is twice its radius (the Roche radius), and their masses increase with distance starting from this point (Fig. 1A). Moreover, in the frame of a circumplanetary gas disk,

Uranus’ satellites should orbit in the ecliptic plane, and not the equatorial plane of the tilted planet (5). Also, Uranus and Neptune might be too light to have retained a massive enough gaseous disk (6). These considerations suggest that an alternative model is needed, to explain at least the origin of the giant planets’ innermost satellites.

Here, we consider a disk of solid material around a planet, similar to Saturn’s rings, where-in planetary tides prevent aggregation within the Roche radius r_R (7) [supplementary text 1 (SM 1)]. It is known that such a tidal disk will spread (8). Thus, the normalized disk lifetime can be defined by $\tau_{\text{disk}} = M_{\text{disk}}/FT_R$, where F is the mass flow through r_R , T_R is the orbital period at r_R , and $M_{\text{disk}} = \pi\Sigma r_R^2$ is the disk’s mass (Σ being the surface density). Using a prescription for the viscosity based on self-gravity and mutual collisions (9), one finds

$$\tau_{\text{disk}} = 0.0425/D^2 \quad (1)$$

where $D = M_{\text{disk}}/M_p$ and M_p is the planet’s mass (SM 2.2.1).

As material migrates beyond r_R , new moons form (10, 11). They are then repelled by the tidal disk through resonant angular momentum exchange and migrate outward as they grow. A satellite of mass M orbiting outside a tidal disk experiences a positive gravitational torque (12)

$$\Gamma = (32\pi^2/27)q^2\Sigma r_R^4 T_R^{-2} \Delta^{-3} \quad (2)$$

where $q = M/M_p$, $\Delta = (r - r_R)/r_R$, and r is the orbital radius. Thus, it migrates outward at a rate (SM 2.1)

$$d\Delta/dt = (2^5/3^3)qDT_R^{-1}\Delta^{-3} \quad (3)$$

The migration rate increases with mass-ratio q and decreases with distance Δ . Based on the restricted three-body problem, we assume that a satellite accretes everything within 2 Hill radii, r_H , from its orbit (13, 14) [$r_H = r(q/3)^{1/3}$]. Thus, as a tidal disk spreads, a competition takes place between accretion and migration. We assume that the satellites do not perturb each other’s orbit or the disk (SM 10), that $\Delta \ll 1$, and that D and τ_{disk} (thus F and Σ) are constant. We find from our analytical model (SM) that moon accretion proceeds in three steps, corresponding to three different regimes of accretion.

When the disk starts spreading, a single moon forms at the disk’s edge (moon 1). As long as $\Delta < 2r_H/r$, it will directly accrete the material flowing through r_R and grow linearly with time, while migrating outward. This is the “continuous regime” (Fig. 2A, SM 3). Integrating Eq. 3 with $M = Ft$, one finds that the condition $\Delta < 2r_H/r$ holds until

$$\Delta = \Delta_c = (3/\tau_{\text{disk}})^{1/2} \quad (4)$$

$$q = q_c = \sim 2\tau_{\text{disk}}^{-3/2}$$

Using Eq. 1, one finds $\Delta_c = 8.4D$, occurring after ~ 10 orbits (SM 3.1).

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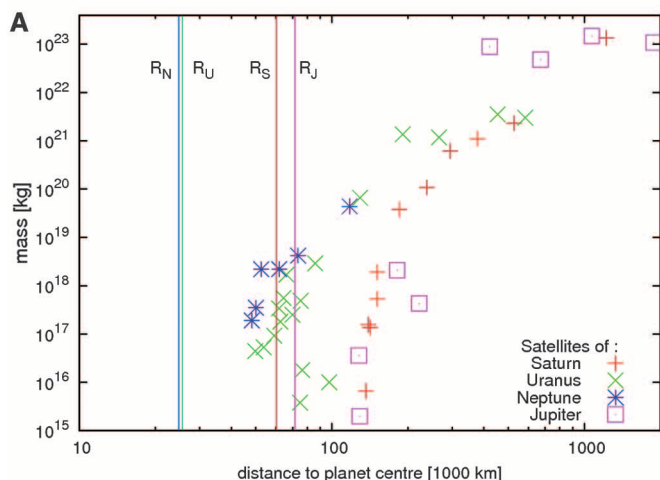
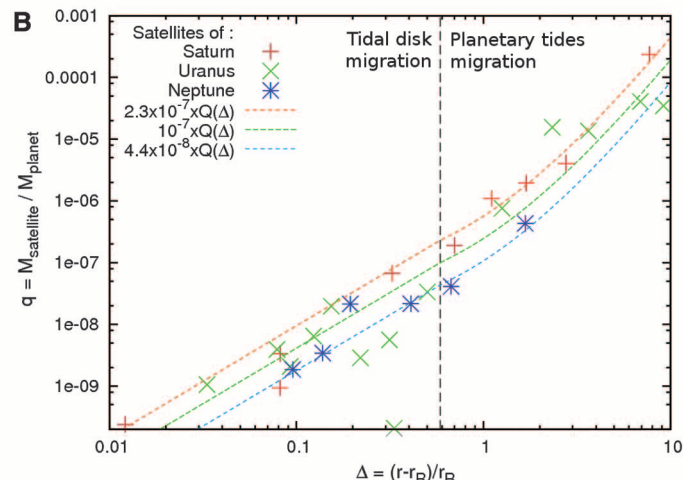
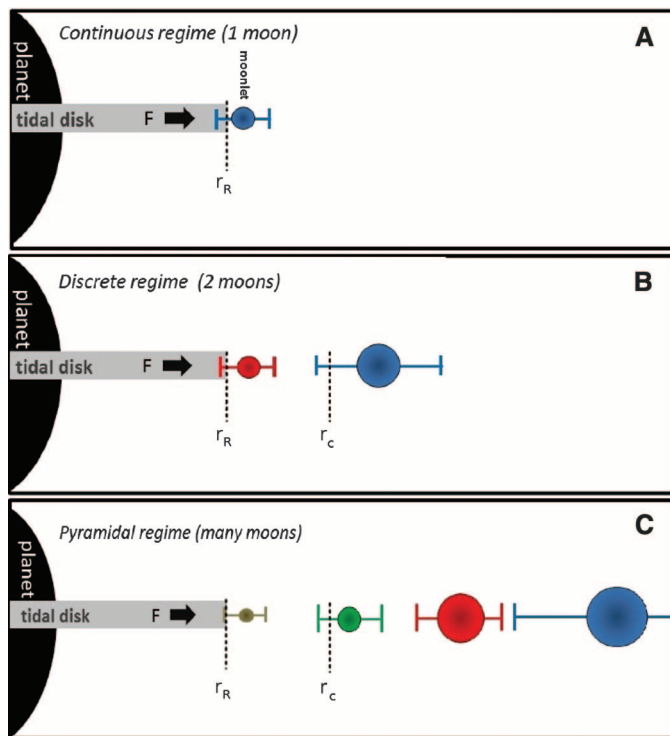


Fig. 1. Distribution of the regular satellites of the giant planets. Saturn: 9 satellites from Pandora to Titan. Uranus: (A) 18 from Cordelia to Oberon; (B) 14 satellites, from Bianca to Oberon (except Cupid and Mab, out of scale). Neptune: Naiad, Thalassa, Despina, Galatea, Larissa, and Proteus. Jupiter: Metis, Adrastea, Amalthea, These, Io, Europa, Ganymede, and Callisto. (A) Mass as a function of the orbital radius. The four systems do not extend all the way down to the planetary radius (vertical line), but a pile-up of small satellites is observed at a specific distance (the Roche limit, r_R). The mass increases from zero with the



distance to r_R , not to the center of the planets. (B) Satellite-to-planet mass ratio q as a function of $\Delta = (r - r_R)/r_R$. The Roche radius for each planet is taken consistently with the mean density of satellites, or with the orbit of the closest one (SM 1). For Saturn, Uranus, and Neptune, $r_R = 140,000, 57,300$, and $44,000$ km, respectively. Short dashed curves: our model for the pyramidal regime $Q(\Delta)$ (eq. S25, SM 6.3): for $\Delta < \Delta_{2:1}$, $q \propto \Delta^{95}$; for $\Delta > \Delta_{2:1}$, $q \propto (\Delta + 1)^{3.9}$, where $\Delta_{2:1} = 0.59$ is marked by the vertical dashed line. Jupiter's system does not fit well and is not shown (SM 7.3).

Fig. 2. Sketches of the three accretion regimes, where the accretion regions are defined as $\pm 2r_H$ around a moon's location. The tidal disk is in gray with F denoting the mass flow at the edge. The first moon to form is in blue, the second in red, and the third in green. (A) Continuous regime: only one moon is present, directly fed by the disk's mass flow. (B) Discrete regime: When the first moon has $r > r_c = r_R(\Delta_c + 1)$, a new moonlet forms (in red). The first moon (blue) continues to grow by accreting these moonlets (red), which are fed by the disk and have masses $\leq M_c$. (C) Pyramidal regime: When the first moon has $r - 2r_H > r_c$, several moons can form and grow up to $M \geq M_c$. Moons with $r - 2r_H > r_c$ grow through merging events between moons of similar masses.



Then, a second moon forms at r_R (moon 2). Moon 2 migrates away rapidly, approaches moon 1, and is caught by it. Another moon forms, which is also eventually accreted by moon 1, and so on. The growth of moon 1 thus proceeds at the same average rate as before, but step by step through the accretion of moonlets: This is the “discrete regime” (Fig. 2B, SM 4 and 5). When $\Delta > \Delta_c +$

$2r_H/r$, moon 1 is too far away to accrete a newly formed moon before this new moon leaves the continuous regime (SM 4.1). This corresponds to

$$\Delta = \Delta_d \sim 3.1\Delta_c \quad (5)$$

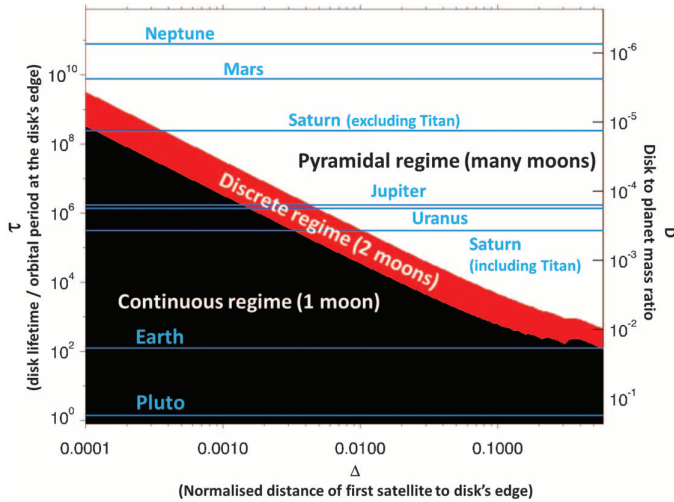
and $q = q_d \sim 20\tau_{\text{disk}}^{-3/2} \sim 2200D^3$ as shown in SM 4. Equation 1 implies that this occurs after

~ 100 orbits. Also, $q_d < 0.1D$ provided $D < 6.7 \times 10^{-3}$. This is always the case around giant planets (see below and SM 7), and it justifies the assumption that D and τ_{disk} are constant. After Δ_d is reached, a third moon appears in the system, and the discrete regime ends.

As moons of fixed mass (produced by the discrete regime) appear successively at a given radius, they migrate outward with decreasing rate, and hence their mutual distance decreases; eventually, they merge. Therefore, moons of double mass are periodically formed, migrate outward, merge again, and so on. Assuming that the satellites do not perturb each other's orbit, mergers occur hierarchically—this is the “pyramidal regime” (SM 6). The moons' masses increase with distance, and an ordered orbital architecture settles. In the region $r < r_{2:1} = 2^{2/3}r_R$, the migration is controlled by the disk's torque (Eq. 3); then the mass-distance relation follows $M \propto \Delta^{1.8}$, and the number density of moons is proportional to $1/\Delta$ (SM 6.1). Consequently, just outside r_R , an accumulation of small moons is expected, consistent with observations (Fig. 1A). Beyond $r_{2:1}$, the migration is controlled by the planet's tides and $M \propto r^{3.9}$ (SM 6.2).

This specific architecture is a testable observational signature of this process. A comparison with today's giant planet systems of regular moons reveals a very good match for Saturn, Uranus, and Neptune (Fig. 1B). Neptune's inner moons (blue stars) match our model (blue dotted line) very well, except for Despina, whose mass is underpredicted by a factor of 3. Uranus' satellites are somewhat more scattered, but the system globally follows our model on the two sides of $r_{2:1}$ (SM 7.4), and the large number of

Fig. 3. Zones of the three regimes in the $\Delta - \tau_{\text{disk}}$ space obtained through numerical integration of the exact disk's torque (SM 8). The right vertical axis displays the corresponding D to τ_{disk} through Eq. 1. When a disk spreads and forms satellites, a satellite follows a horizontal line (Δ increases and τ_{disk} is constant), from left to right. First, the satellite appears in the continuous regime (black region), where it is fed directly from the disk, while migrating away. Then it enters the discrete regime (red region) where it grows by regularly accreting new moons appearing at the disk's edge, in the black region. Finally, it leaves the discrete regime, and many satellites may form and accrete all together—this is the pyramidal regime (white region). The boundaries between the regions follow exactly our analytical expressions for small Δ (Eqs. 4 and 5). Refined equations for the boundaries are provided in SM 9. The horizontal lines show the path that may have been followed around the solar system's planets. The corresponding values of D were computed in SM 7 and for Earth, Jupiter, Saturn, Uranus, and Neptune are 0.018, 1.6×10^{-4} , 1.3×10^{-5} , 1.7×10^{-4} , and 7.3×10^{-7} , respectively.



satellites outside r_R appears to be a natural by-product of the pyramidal regime; however, the four main bodies are not ranked by mass. Saturn's case is especially notable because the eight regular satellites from Pandora to Titan (considering Janus plus Epimetheus as one object) have masses that closely follow our model (red dashed line), through four orders of magnitude in distance and six in mass. Although unlikely, given our present understanding of Saturn's tides (15), this result suggests that even Titan might have formed from the spreading of Saturn's initially massive rings. Titan being about 50 times more massive than Rhea could be understood in the frame of our model if the rings were initially very massive ($D \sim 10^{-3}$, see below), favoring the formation of one dominant moon, which migrated fast outward, taking most of the mass. Then, as the mass of the rings decreased, a standard pyramidal regime took place, giving birth to the other moons. However, Titan's tidal age (the time needed to reach its orbit through Saturn's tides) is estimated at about 10 billion years (11); thus, Titan's fit with our model may be a coincidence.

Jupiter's system isn't compatible with the pyramidal regime (SM 7.3), suggesting a different formation mechanism, even if the Laplace resonance may have erased the initial configuration. The system of the Galilean moons is satisfactorily explained by the circumplanetary disk models (1–4). These models may also explain the formation of Saturn's moons Titan and Iapetus but not those of the other satellites. Indeed, the conditions inside Saturn's circumplanetary disc were very different. Jupiter opened up a gap in the protoplanetary nebula early in the history of

the solar system, whereas Saturn, which formed later and is less massive, hardly did (16).

Our model also predicts the conditions under which one moon is formed rather than numerous ones: Surprisingly, the mass and distance of moon 1 at the end of the discrete regime depend on only one parameter, τ_{disk} (or D). Figure 3 shows the different regimes encountered during the recession of a moon in the $(\Delta, \tau_{\text{disk}})$ space (SM 8). When τ_{disk} is large, a moon that forms in the discrete regime reaches a low mass and small Δ , as shown by Eq. 5. Thus, the pyramidal regime dominates, where many moons coexist and migrate away. For each of the solar system's planets, the mass of the putative tidal disk is chosen to be about 1.5 times the mass of its current satellite system (SM 7) (Fig. 3). For all the giant planets, $D < 2 \times 10^{-4}$, so that $\tau_{\text{disk}} > 10^6$, making the pyramidal regime the final outcome, which explains the presence and the distribution of their numerous innermost regular satellites.

Conversely, when τ_{disk} is short, the continuous and discrete regimes are more efficient, and thus a massive satellite is built and migrates to large distances (17). This applies to Pluto and Earth, which have only one moon. Our model agrees well with N -body numerical simulations of the formation of Charon and the Moon (10, 18–20) but neglects thermodynamical effects. This limitation is investigated in SM 7.1. In this case, where a single moon forms as the disk spreads, D varies strongly with time, making Eqs. 4 and 5 inaccurate. The system can still be solved provided F is constant, which is likely in a disk at thermodynamical equilibrium. The Earth's Moon probably finished its accretion in the discrete regime, allowing the possibility

of a short-lived and low-mass companion moon. As the companion approached the proto-Moon, it may have been trapped in a horseshoe orbit, and later impacted the proto-Moon, as was recently suggested to explain the Moon's highlands (21).

These results strongly suggest that, like the Moon and Charon, most regular satellites of Saturn, Uranus, and Neptune formed from the spreading of a tidal disk. It may be that only the giant planets' most massive regular satellites (the Galilean moons, Titan, and Iapetus) formed directly from the planet's subnebula (1–4). Many models have been proposed for the formation of the giant planets' massive rings. Rings could be remnants of disrupted satellites [either by an impact (22, 23) or by tides (24)], an explanation that is favored for Saturn, or remnants from tidally disrupted comets (23, 25), which is more likely for Uranus and Neptune (23). Uranus' and Neptune's large inclinations indicate that giant impacts capable of generating massive rings were common on the ice giants during the formation of the solar system (26). However, the many uncertainties in the initial conditions of giant planet formation make it hard to conclude how massive rings formed. The way Uranus' and Neptune's massive rings disappeared is also an open question. Viscous spreading by itself would not be efficient enough to make the rings disappear because it becomes increasingly inefficient when the disk loses mass (8). Atmospheric gas drag (22, 27), the Yarkovsky effect (28), or slow grinding of the rings through meteoritic bombardment (22) could all be invoked, but their role is still not well understood. However, our model is compatible with existing ring-formation scenarios, and thus the most common mechanism of satellite formation is likely to be the spreading of a tidal disk surrounding a planet, terrestrial or giant, besides other processes (1–4). The structure of the satellite system then depends only on the disk's lifetime.

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Chemically and Geographically Distinct Solid-Phase Iron Pools in the Southern Ocean

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Iron is a limiting nutrient in many parts of the oceans, including the unproductive regions of the Southern Ocean. Although the dominant fraction of the marine iron pool occurs in the form of solid-phase particles, its chemical speciation and mineralogy are challenging to characterize on a regional scale. We describe a diverse array of iron particles, ranging from 20 to 700 nanometers in diameter, in the waters of the Southern Ocean euphotic zone. Distinct variations in the oxidation state and composition of these iron particles exist between the coasts of South Africa and Antarctica, with different iron pools occurring in different frontal zones. These speciation variations can result in solubility differences that may affect the production of bioavailable dissolved iron.

Dissolved, or soluble, iron plays a central role in vital phytoplankton cellular processes, including photosynthesis and the

coupled uptake of CO₂ in oceans (1, 2). The concentration of soluble Fe is strongly linked to ocean primary productivity. However, the solubility of Fe is low in oxygenated seawater; the majority of Fe in oceans exists in the form of nanoparticles (<0.2 μm) and biogenic and lithogenic colloids (>0.2 μm). This particulate-bound Fe constitutes as much as 65 to 85% of the total dissolvable Fe fraction in the mixed layer of the Southern Ocean (3–5). Considering the magnitude of this particulate-bound Fe pool,

which is an important reserve source of Fe for photosynthetic organisms, the observation of Fe limitation in certain parts of the Southern Ocean is surprising.

Both the solubility and the bioavailability of particulate-bound Fe vary according to differences in Fe oxidation state, mineralogy, crystallinity, structural impurities (e.g., Al), and the structure and concentration of dissolved organic ligands (6–8). To improve our understanding of Fe limitation in the Southern Ocean, we examined the structure and chemistry of Fe particles from water samples collected on three different scientific cruises: South African National Antarctic Expedition (SANAE) 49, SANAE 50, and GEOTRACES D357 (5). This strategy ensured samples across the fronts of the Southern Ocean, which distinguish water masses with different hydrographic characteristics. The distribution of Fe in particles as a function of particle size was analyzed by collecting high-resolution Fe maps (resolution ~12 nm) at the Fe L₃-absorption edge (~710 eV) (Fig. 1). The oxidation state and coordination environment of Fe in particles were analyzed by collecting Fe L₃-edge x-ray absorption near-edge structure (XANES) spectra. We also measured the association of Fe with Al, a solubility modifier and source indicator. The distribution and chemistry of Al was similarly analyzed using Al maps and XANES spectra at the Al K-edge (~1570 eV).

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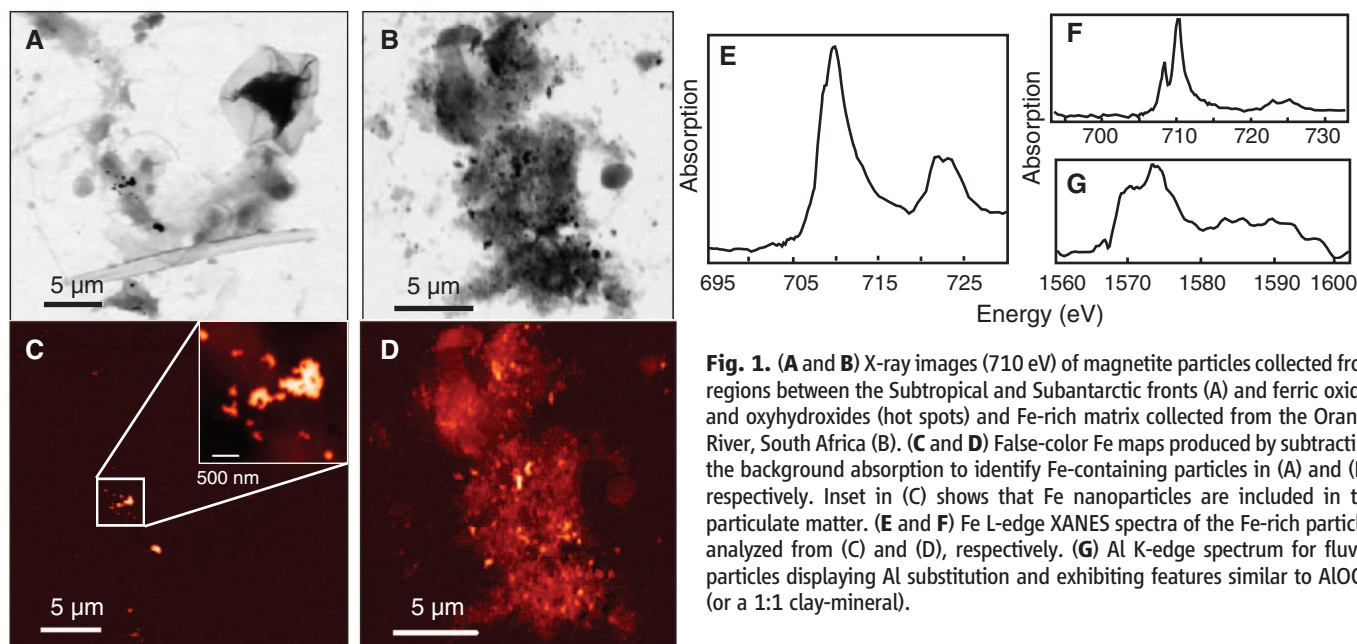


Fig. 1. (A and B) X-ray images (710 eV) of magnetite particles collected from regions between the Subtropical and Subantarctic fronts (A) and ferric oxides and oxyhydroxides (hot spots) and Fe-rich matrix collected from the Orange River, South Africa (B). (C and D) False-color Fe maps produced by subtracting the background absorption to identify Fe-containing particles in (A) and (B), respectively. Inset in (C) shows that Fe nanoparticles are included in the particulate matter. (E and F) Fe L-edge XANES spectra of the Fe-rich particles analyzed from (C) and (D), respectively. (G) Al K-edge spectrum for fluvial particles displaying Al substitution and exhibiting features similar to AlOOH (or a 1:1 clay-mineral).

All samples were analyzed at the Advanced Light Source x-ray spectromicroscopy facility, which allows collection of images and XANES spectra at a resolution of 12 nm under ambient conditions (9).

The Fe L_3 -edge XANES spectra are sensitive to the oxidation state and the coordination environment of Fe (10, 11). Specifically, the energy differences (ΔE) between the two main transitions and their intensity ratios can be used to distinguish Fe(II) phases from Fe(III) phases and to discern variations in the coordination environment for each oxidation state (Fig. 2). On the basis of their ΔE and intensity ratio values in the L_3 edge, we categorize the Fe-rich phases into five distinct chemical classes: pure Fe(III) species (including oxyhydroxides; purity refers to valence state and not speciation), pure Fe(II) species, magnetite, and two mixed-valence phases that show a more varied distribution on the spectral plot (Fig. 2). Although constituent mineral phases in Fe particles can be identified using the Fe L_3 -edge spectra (fig. S1), we have limited our classification to these five broad classes because mineral identification in natural samples is complicated by the presence of chemical impurities, surface coatings, and poor crystallinity.

The Fe maps of particulate matter from the Southern Ocean are largely characterized by single isolated particles; where present, the surrounding organic and mineral matrix is conspicuously poor in Fe (Fig. 1, A and C). The discrete particles are 20 to 700 nm in diameter, with a mean size of ~200 nm. Larger particles, exceeding sizes of 500 nm, are much less common and are uniquely confined to sites near continental and island shelves. Although an apparent correlation between the size of particles and their Fe valence state and coordination environment was absent, the pure Fe(II) phases are generally larger and have spectral features indicative of greater variability in their Fe coordination environment. The chemistry of mixed-valence phases is not uniform, and their spectra do not correlate to those of known Fe minerals (fig. S1). This suggests the presence of mineral mixtures and structural heterogeneity, possibly caused by the presence of Fe(II) and Fe(III) amorphous phases and/or binding to inorganic and organic ligands. In contrast to the discrete nature and sparse distribution of these marine particles, fluvial Fe-rich particles in streams draining into the South Atlantic Ocean (Orange, Olifants, and Berg rivers) are much more abundant, larger (20 nm to 5 μ m), and are commonly associated with Fe-rich inorganic and organic matrices (Fig. 1, B and D).

Iron associated with solid phases displayed speciation variation along the two transects sampled—one between SANAE (coast of Queen Maud Land, Antarctica) and Cape Town, the other between SANAE and South Georgia Island (Fig. 3). Samples from north of the Subtropical Front (i.e., those most proximal to the African continent) show the largest degree of chemical heterogeneity, consisting of predominantly Fe(III)-rich par-

ticles with substantial Al association. The water mass between the Subtropical and Subantarctic fronts, which receives relatively high atmospheric dust inputs from Patagonia (12), is overwhelmingly dominated by magnetite. Between the Subantarctic Front and the Southern Boundary where deep circumpolar waters upwell (13, 14), Fe particles predominantly contain Fe(III) phases. The

Fe(III) phases in these frontal zones display x-ray spectral features more characteristic of Fe(III) oxyhydroxides than Fe(III) oxides (Fig. 2 and fig. S1). In contrast, samples from south of the Southern Boundary, in the Weddell Sea gyre, are largely composed of particles rich in Fe(II). Such a high abundance of Fe(II)-rich particles and magnetite in the oxygen-rich photic zone is surprising,

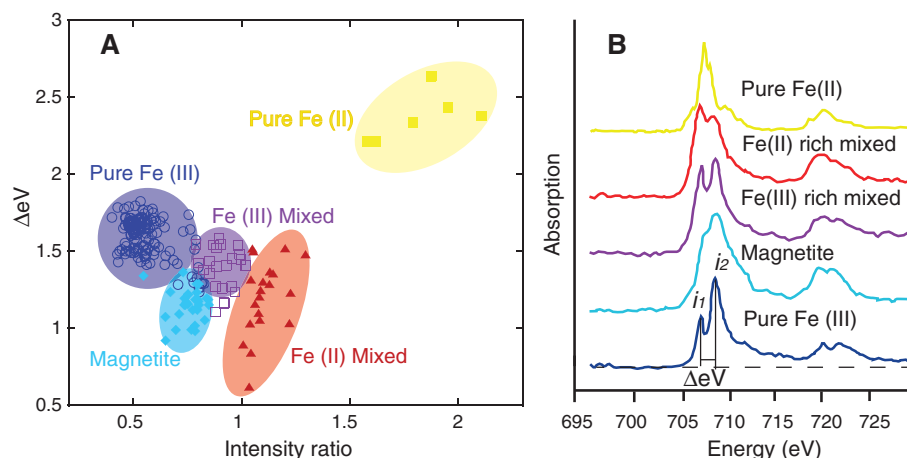


Fig. 2. (A) Iron particle speciation plotted and defined according to the particles' spectral features. Pure Fe(III), pure Fe(II), and magnetite phases occupy discrete fields; the mixed-valence species are distinguished by their variations in spectral intensity ratios. (B) Generalized Fe L-edge XANES spectra of the five species identified in the South Atlantic and Southern oceans; colors correspond to fields in (A). The ΔE value is calculated as the energy difference between peaks i_1 and i_2 ; the intensity ratio value is given as absorption intensity i_1/i_2 .

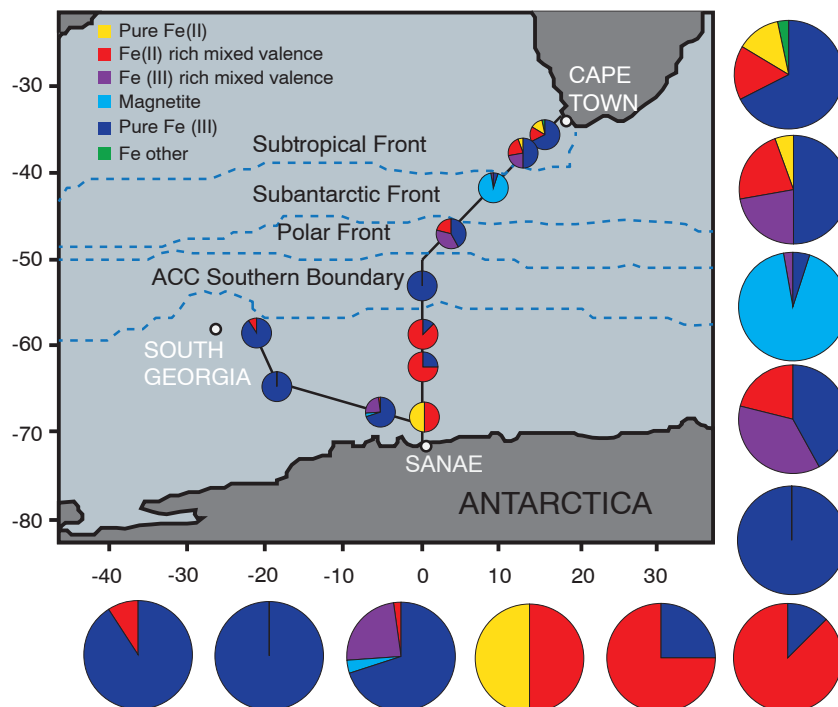


Fig. 3. Surface water transects (SANAE 49 and SANAE 50) of the South Atlantic and Southern oceans, and Fe speciation along the cruise transects. Sampling was conducted at different locations on these transects so as to ensure complete spatial resolution between the pertinent oceanic fronts. The pie charts (and their enlarged counterparts) show the relative contribution of Fe species, as defined by their spectral features, in the solid-phase Fe pool at each site. The Antarctic Circumpolar Current (ACC) Southern Boundary is defined as the southern terminus of the Upper Circumpolar Deep Water (13).

and additional x-ray spectroscopy measurements at the aluminum, silicon (~1840 eV), and carbon (~285 eV) edges show that these phases are not ferrous aluminosilicates and that they exhibit strong association with organic carbon. Possible contributors to the stability of these Fe(II) particles are the presence of thick organic coatings (or organic complexes) on their surfaces, photochemical reduction of Fe(III), structural impurities, and the slower oxidation rate of Fe(II) at low temperatures.

We also found Fe(III)-rich particles within the Southern Boundary front on the SANAÉ–South Georgia Island transect. The differences in Fe speciation between the two transects off the Antarctic shelf may be caused by differences in Fe sources, with westward samples likely displaying island effects from the upstream South Sandwich Island chain. Although not extensively examined, variable speciation with water column depth was observed on the basis of a single depth profile collected beyond the continental slope of southern Africa, suggesting internal recycling and possible differences in biological processing and Fe sources at different depths (fig. S2).

The association of Al [a well-known solubility modifier of Fe(III) minerals] with Fe in particles also showed variations along the two transects (Fig. 4). Aluminum substitution for Fe(III) in Fe oxides is commonly encountered in weathered material in the pedosphere, where Al/Fe ratios range between 0 and 0.47 for soil goethite (15) and amorphous phases often display higher values. We found that surface Fe par-

ticles collected nearer to and downstream of continental and island arc shelves, with the exception of the Antarctic shelf where Fe(II) phases predominate, typically exhibit relatively high Al/Fe ratios between 0.01 and 0.27. Off the African coast, the Al/Fe ratio increased with distance away from the fluvial source before dropping to lower values in the open ocean (Fig. 4). The Antarctic shelf showed predominantly Fe(II)-rich phases whose Al/Fe ratios were low or undetectable because of limited substitution of Al for Fe(II). Where depth samples were analyzed, the Al/Fe ratios were much higher (~0.17) at depth relative to the corresponding surface values (~0.08)—possibly a consequence of the solubility-depressing effect of Al substitution on sinking Fe particles, which would preserve only the most recalcitrant Al-rich phases from mineralization.

Laboratory studies have indicated that the biological availability of Fe is strongly linked to its solubility, which is influenced by chemical speciation [Fe(II) is more soluble (e.g., south of the Southern Boundary)], mineralogy (amorphous phases are more soluble), and Al substitution in Fe(III) oxides [Al-rich Fe phases are less soluble (e.g., the subtropical domain)]. Because all of these Fe forms exist in different abundances in different parts of the Southern Ocean, variation in the bioavailability of the Fe pool should be influenced by these factors. In addition, heterogeneity in the surface chemistry of the different Fe particles found in the water column is expected to affect the ability of microorganisms to scavenge Fe and other essential trace elements such as Cu

and Zn, which are strongly associated with the Fe particle surfaces (16).

Sustained primary productivity requires the constant replenishment of the truly dissolved bioavailable Fe pool, which is continually being used by ambient phytoplankton species. One mechanism for this replenishment is by dissolution of the solid-phase Fe pool. Given that Fe solubility for the relatively simple Fe oxide and oxyhydroxide system varies over three orders of magnitude (8), our findings on heterogeneity in Fe particle chemistry imply that the solubility and expected dissolution rates of Fe particles vary regionally in the Southern Ocean. The causes of the observed heterogeneity in Fe speciation and its implications for Fe bioavailability are uncertain. However, a comparison between the average summertime chlorophyll *a* concentration and the abundance and distribution of labile Fe forms [for example, Fe(II)] reveals trends (5) (figs. S3 to S5) that allude to the effect of Fe speciation on biology, and vice versa.

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Supplementary Materials

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Materials and Methods

Supplementary Text

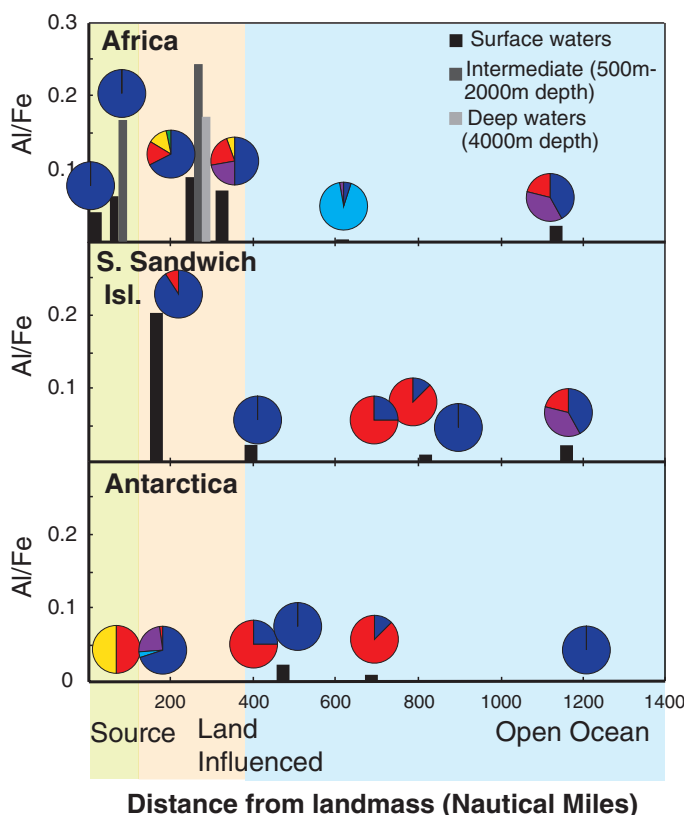
Figs. S1 to S5

Table S1

References (18–53)

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Fig. 4. Variations in the Al/Fe ratio of Fe particles with increasing distance from land mass and with depth. Source regions include fluvial inputs from the African river systems described in the text as well as marine sites located on the respective continental shelves. Land-influenced sites, which typically show higher Al/Fe ratios, are potentially influenced by continental and shelf sources. They are designated taking into account the vectors of ocean current movement and are all located within distances previously reported for continental Fe supply (17). Iron speciation pie charts are included to match the Al/Fe data to the sampling sites shown in Fig. 3.



A Large-Scale Model of the Functioning Brain

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A central challenge for cognitive and systems neuroscience is to relate the incredibly complex behavior of animals to the equally complex activity of their brains. Recently described, large-scale neural models have not bridged this gap between neural activity and biological function. In this work, we present a 2.5-million-neuron model of the brain (called “Spaun”) that bridges this gap by exhibiting many different behaviors. The model is presented only with visual image sequences, and it draws all of its responses with a physically modeled arm. Although simplified, the model captures many aspects of neuroanatomy, neurophysiology, and psychological behavior, which we demonstrate via eight diverse tasks.

Large-scale neural simulations are becoming increasingly common [see (1) for a review]. These include the ambitious Blue Brain Project (2), which has simulated about 1 million neurons in cortical columns and includes considerable biological detail, accurately reflecting spatial structure, connectivity statistics, and other neural properties. More recent work has simulated many more neurons, such as the 1 billion neurons simulated in the Cognitive Computation Project (3), which has been hailed as a cat-scale simulation. A human-scale simulation of 100 billion neurons has also been reported (4).

Although impressive scaling has been achieved, no previous large-scale spiking neuron models have demonstrated how such simulations connect to a variety of specific observable behaviors. The focus of this past work has been on scaling to larger numbers of neurons and more detailed neuron models. Unfortunately, simulating a complex brain alone does not address one of the central challenges for neuroscience: explaining how complex brain activity generates complex behavior. In contrast, we present here a spiking neuron model of 2.5 million neurons that is centrally directed to bridging the brain-behavior gap. Our model embodies neuroanatomical and neurophysiological constraints, making it directly comparable to neural data at many levels of analysis. Critically, the model can perform a wide variety of behaviorally relevant functions. We show results on eight different tasks that are performed by the same model, without modification.

All inputs to the model are 28 by 28 images of handwritten or typed characters. All outputs are the movements of a physically modeled arm that has mass, length, inertia, etc. For convenience, we refer to the model as “Spaun” (Semantic Pointer Architecture Unified Network) (see Fig. 1 and supplementary materials and methods section S1.1). Many of the tasks we have chosen are the subject of extensive modeling in their own right [e.g., image recognition (5, 6), serial working memory (WM) (7, 8), and reinforcement learning (RL) (9, 10)], and others demonstrate abilities that are rare for

neural network research and have not yet been demonstrated in spiking networks (e.g., counting, question answering, rapid variable creation, and fluid reasoning). The eight tasks (termed “A0” to “A7”) that Spaun performs are: (A0) Copy drawing. Given a randomly chosen handwritten digit, Spaun should produce the same digit written in the same style as the handwriting (movie S1; all supplemental movies can be viewed at <http://nengo.ca/build-a-brain/spaunvideos>). (A1) Image recognition. Given a randomly chosen handwritten digit, Spaun should produce the same digit written in its default writing (movie S2). (A2) RL. Spaun should perform a three-armed bandit task, in which it must determine which of three possible choices generates the greatest stochastically generated reward. Reward contingencies can change from trial to trial (movie S3). (A3) Serial WM. Given a list of any length, Spaun should reproduce it (movie S4). (A4) Counting. Given a starting value and a count value, Spaun should write the final value (that is, the sum of the two values) (movie S5). (A5) Question answering. Given a list of numbers, Spaun should answer either one of two possible questions: (i) what is in a given position in the list? (a “P” question) or (ii) given a kind of number, at what position is this number in the list? (a “K” question) (movie S6). (A6) Rapid variable creation. Given example syntactic input/output patterns (e.g., 0 0 7 4 → 7 4; 0 0 2 4 → 2 4; etc.), Spaun should complete a novel pattern given only the input (e.g., 0 0 1 4 → ?) (movie S7). (A7) Fluid reasoning. Spaun should perform a syntactic or semantic reasoning task that is isomorphic to the induction problems from the Raven’s Progressive Matrices (RPM) test for fluid intelligence (11). This task requires completing patterns of the form: 1 2 3; 5 6 7; 3 4 ? (movie S8). Each input image is shown for 150 ms and separated by a 150-ms blank (see table S2 for example inputs for each task). The model is told what the task will be by showing it an “A” and the number of the task (0 to 7). The model is then shown input defining the task (see Figs. 2 and 3 for examples). Spaun is robust to invalid input (fig. S10) and performs tasks in any order without modeler intervention.

Figure 1A shows the anatomical architecture of the model. Connectivity and functional ascriptions to brain areas in Spaun are consistent with current empirical evidence (table S1). In general,

we modeled neuron and synaptic response properties on the electrophysiology literature for the relevant anatomical areas. For instance, the basal ganglia have largely GABAergic neurons, with dopamine modulating learning in the striatum, and the cortex has largely *N*-methyl-D-aspartate and AMPA synaptic connections (supplementary section S1.3). As a result, the dynamics in the model are tightly constrained by underlying neural properties (see supplementary section S2.4).

The functional architecture of the model is described in Fig. 1B. The network implementing the Spaun model consists of three compression hierarchies, an action-selection mechanism, and five subsystems. Components of the model communicate using spiking neurons that implement neural representations that we call “semantic pointers,” using various firing patterns. Semantic pointers can be understood as being elements of a compressed neural vector space (supplementary sections S1.1 and S1.2). Compression is a natural way to understand much of neural processing. For instance, the number of cells in the visual hierarchy gradually decreases from the primary visual cortex (V1) to the inferior temporal cortex (IT) (12), meaning that the information has been compressed from a higher-dimensional (image-based) space into a lower-dimensional (feature) space (supplementary section S1.3). This same kind of operation maps well to the motor hierarchy (13), where lower-dimensional firing patterns are successively decompressed (for example, when a lower-dimensional motor representation in Euclidean space moves down the motor hierarchy to higher-dimensional muscle space).

Compression is functionally important because low-dimensional representations can be more efficiently manipulated for a variety of neural computations. Consequently, learning or defining different compression/decompression operations provides a means of generating neural representations that are well suited to a variety of neural computations. The specific compression hierarchies in Spaun are (see Fig. 1B): (i) a visual hierarchy, which compresses image input into lower-dimensional firing patterns; (ii) a motor hierarchy that decompresses firing patterns in a low-dimensional space to drive a simulated arm; and (iii) a WM, which constructs compressed firing patterns to store serial position information. The WM subsystem includes several subcomponents that provide stable representations of intermediate task states, task subgoals, and context.

Spaun’s action-selection mechanism is based on a spiking basal ganglia model that we have developed in other work (14) but is here extended to process higher-dimensional neural representations. The basal ganglia determine which state the network should be in, switching as appropriate for the current task goals. Consequently, the model’s functional states are not hardwired, as the basal ganglia are able to control the order of operations by changing information flow between subsystems of the architecture.

The five subsystems, from left to right in Fig. 1B, are used to: (i) map the visual hierarchy firing pattern to a conceptual firing pattern as needed

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(information encoding), (ii) extract relations between input elements (transformation calculation), (iii) evaluate the reward associated with the input (reward evaluation), (iv) decompress firing patterns from memory to conceptual firing pattern (information decoding), and (v) map conceptual firing patterns to motor firing patterns and control motor timing (motor processing). Supplementary materials section S1.3 includes a more detailed description of each element. It is critical to note that the elements of Spaun are not task-specific. That is, they are used in a variety of combinations to perform the chosen tasks, resulting in the same circuitry being used across tasks. This makes it straightforward to extend the model to some new tasks (supplementary section S2.4).

The neural connection weights of these subsystems can be learned with a biologically plausible spike-based rule (15), although we use more efficient optimization methods to determine the synaptic weights (supplementary section S1.2).

To help explain the functioning of the model, we consider the serial WM task. Figure 2A shows the information flow through the model for this task. The storage and recall states of the network are common to many tasks. For the WM task, these states occur immediately one after the other, although the delay is task-dependent. Initially, seeing the task identifier (A3) switches Spaun into the storage state. In the storage state, the network compresses the incoming image into a visually based firing pattern (FP in the figure) that encodes vi-

sual features, maps that firing pattern to another firing pattern that represents the related concept (e.g., “TWO”; see supplementary section S1.3), and then compresses that firing pattern into a memory trace that is stored in WM. The compression operator (i.e., “ $\otimes$ ”) binds the concept firing pattern (e.g., TWO) to a position representation (e.g., P3) and adds the result (i.e., TWO $\otimes$ P3, as in Fig. 2C) to WM. As shown in Fig. 2C, this process is repeated as long as items are shown to the model.

Figure 2B shows a screen capture from a movie of the WM simulation. When the model sees the “?” input (as in Fig. 2B), the basal ganglia reroute cortical connectivity to allow Spaun to recall the input stored in the dorsolateral prefrontal cortex (DLPFC). Recall consists of decompressing an item from the stored representation of the full list, mapping the resulting concept vector to a known high-level motor command, and then decompressing that motor command to specific joint torques to move the arm. This process is repeated for each position in the WM, to generate Spaun’s full written response. Figure 2C shows the entire process unfolding over time, including spike rasters, conceptual decodings of the contents of DLPFC, and the input and output.

Critically, no single task captures the distinct features of this model. To highlight the diversity of tasks the model is able to perform, Fig. 3 shows the results of the model performing a low-level perceptual-motor task (the copy-drawing task), as well as a challenging pattern-induction task only performed by humans (the RPM task).

Specifically, Fig. 3A demonstrates that the low-level perceptual features in the input are available to Spaun to drive its motor behavior. Figure 3B demonstrates the RPM task for one sample pattern (see fig. S6 for an additional example). In this task, Spaun is presented with two groups of three related items and must learn the relation between items in the groups. Spaun then uses its inferred relation to complete the pattern of a third set of items. Similarity plots for the DLPFC show conceptual decodings of neural activities. The model learns the relation between subsequent strings of numbers by comparing patterns in DLPFC1 and DLPFC2 (see supplementary section S1.3). Human participants average 89% correct (chance is 13%) on the matrices that include only an induction rule (5 of 36 matrices) (16). Spaun performs similarly, achieving a match-adjusted success rate of 88% (see supplementary section S2.3).

To demonstrate that Spaun captures general psychological features of behavior, it is critical to be able to simulate populations of participants. Every time a specific instance of Spaun is generated, the parameters of the neurons are picked from random distributions (supplementary section S1.4). Consequently, generating many instances allows for comparison with population-wide behavioral data. Figure 4 compares the recall accuracy of the model as a function of list length and position in a serial recall task to human population data. As with human data (17), Spaun produces distinct recency (items at the end are recalled with greater accuracy) and primacy

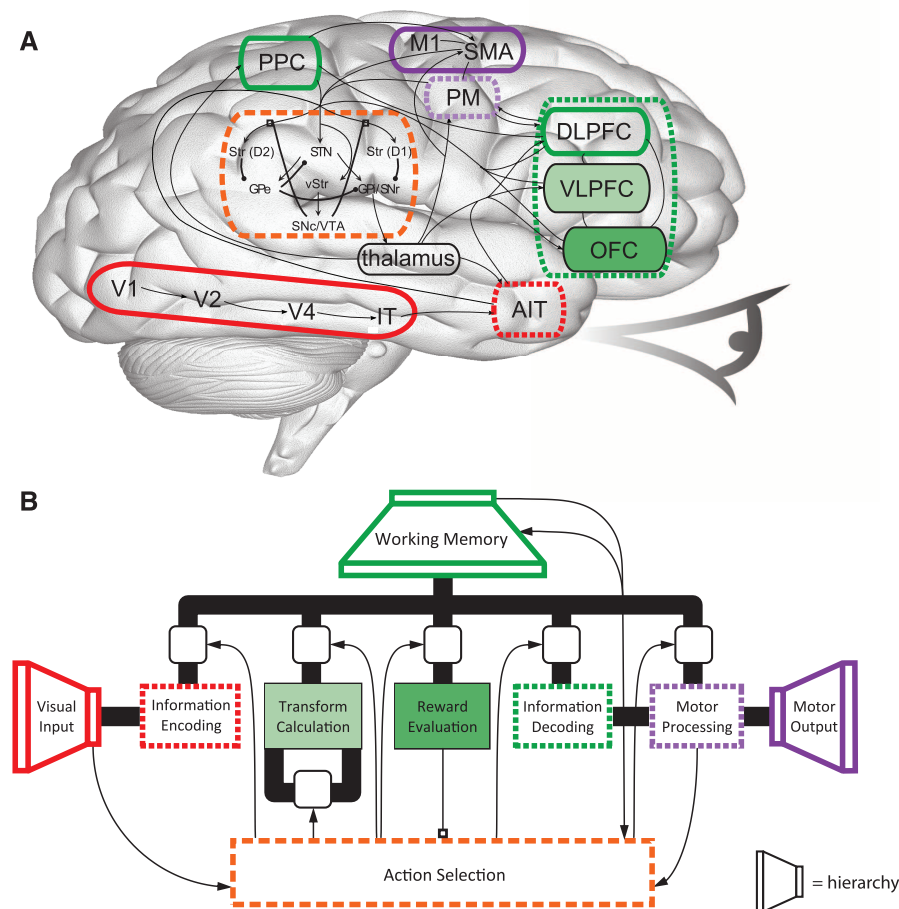


Fig. 1. Anatomical and functional architecture of Spaun. **(A)** The anatomical architecture of Spaun shows the major brain structures included in the model and their connectivity. Lines terminating in circles indicate GABAergic connections. Lines terminating in open squares indicate modulatory dopaminergic connections. Box styles and colors indicate the relationship with the functional architecture in (B). PPC, posterior parietal cortex; M1, primary motor cortex; SMA, supplementary motor area; PM, premotor cortex; VLPFC, ventrolateral prefrontal cortex; OFC, orbitofrontal cortex; AIT, anterior inferior temporal cortex; Str, striatum; vStr, ventral striatum; STN, subthalamic nucleus; GPi, globus pallidus externus; GPi, globus pallidus internus; SNr, substantia nigra pars reticulata; SNc, substantia nigra pars compacta; VTA, ventral tegmental area; V2, secondary visual cortex; V4, extrastriate visual cortex. **(B)** The functional architecture of Spaun. Thick black lines indicate communication between elements of the cortex; thin lines indicate communication between the action-selection mechanism (basal ganglia) and the cortex. Boxes with rounded edges indicate that the action-selection mechanism can use activity changes to manipulate the flow of information into a subsystem. The open-square end of the line connecting reward evaluation and action selection denotes that this connection modulates connection weights. See table S1 for more detailed definitions of abbreviations, a summary of the function to anatomy mapping, and references supporting Spaun’s anatomical and functional assumptions.

(items at the beginning are recalled with greater accuracy) effects. A good match to human data from a rapid serial-memory task using digits and short presentation times (18) is also evident, with 17 of 22 human mean values within the 95% confidence interval of 40 instances of the model. Additional population comparisons are presented in fig. S8.

To this point, we have only described performance on three of the eight tasks that Spaun performs. The tasks not yet discussed are: (i) image recognition, for which the model achieves 94% accuracy on untrained data from the MNIST handwriting database [human accuracy is ~98% (19)]; (ii) RL, for which the model is able to learn reward-dependent actions in a variable environment using known neural mechanisms (fig. S5); (iii) counting, for which the model reproduces human reaction times and scaling of variability (fig. S8A); (iv) question answering, for which the model generates a novel behavioral prediction (fig. S8B); and (v) rapid variable creation, for which the model instantiates the first neural architecture able to solve this challenging task (fig. S11).

However, the central purpose of this work is not to explain any one of these tasks, but to propose a unified set of neural mechanisms able to perform them all. In a sense, the complex task solved by Spaun is one of coordination. That is, the rapid flexibility of biological systems is its target of explanation. The specific dynamics of Spaun's responses to this wide variety of tasks is governed by four parameters, each of which is set empirically (the time constants of the neurotransmitters; see supplementary section S2.4). Thus, without fitting, the model is consistent with dynamics from single cells and behavior (see figs. S8 and S11 and supplementary section S2.4), and is able to switch between a wide variety of tasks quickly and robustly (fig. S10).

Although Spaun's main contribution lies in its breadth, it also embodies new hypotheses regarding how specific tasks are solved. For instance, the proposed method of solving the rapid variable-creation task is distinct to Spaun [this task has been identified as one that no contemporary neural architecture could perform as quickly as humans (20)], as is the account of serial WM. Such hypotheses have resulted in new testable predictions (figs. S8 and S9). Still, Spaun's uniqueness lies in its being a platform for exploring the robust flexibility of biological cognition. Consider the example of learning: Learning in Spaun takes on many forms. Although learning takes place in the RPM, rapid variable-creation, and RL, connection-weight changes only occur in the RL task (supplementary section S2.4). In most neural models, this kind of learning is often used as the main method of model construction, and it is possible to learn all of the elements of the Spaun model in this traditional sense (supplementary section S2.4). However, constructing models in this manner does not address a central, difficult challenge of learning in biological brains. That challenge consists of explaining how robust learning can occur in a continuously operating, complex, and multifunctional brain.

Spaun minimally demonstrates this kind of learning in the RL task, as connection-weight

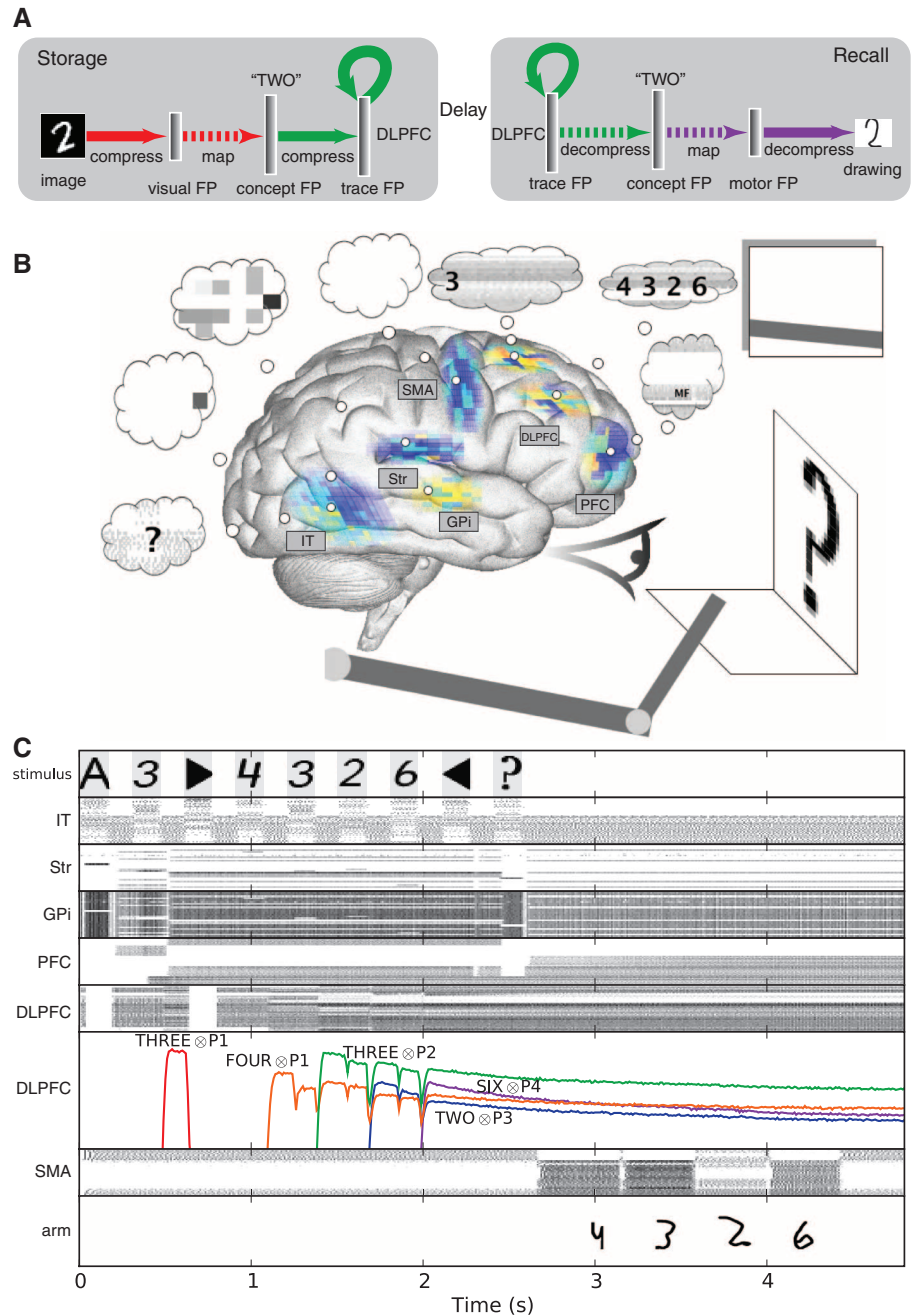


Fig. 2. The serial WM task. (A) Information flow through Spaun during the WM task. Line style and color indicate the element of the functional architecture in Fig. 1B responsible for that function. FP, firing pattern. (B) A screen capture from the simulation movie of this task (supplementary section S2.1), taken at the 2.5-s mark of the time course plot in (C). The input image is on the right, the output is drawn on the surface below the arm. Spatially organized (neurons with similar tuning are near one another), low-pass-filtered neuron activity is approximately mapped to relevant cortical areas and shown in color (red is high activity, blue is low). Thought bubbles show spike trains, and the results of decoding those spikes are in the overlaid text. For Str, the thought bubble shows decoded utilities of possible actions, and in GPI the selected action is darkest. (C) Time course of a single run of the serial WM task. The stimulus row shows input images. The arm row shows digits drawn by Spaun. Other rows are labeled by their anatomical area. Similarity plots (solid colored lines) show the dot product (i.e., similarity) between the decoded representation from the spike raster plot and concepts in Spaun's vocabulary. These plots provide a conceptual decoding of the spiking activity, but this decoding is not used by the model (supplementary section S1.1). Raster plots in this figure are generated by randomly selecting 2000 neurons from the relevant population and discarding any neurons with a variance of less than 10% over the run. $\otimes$ denotes the convolution compression operator.

changes do not adversely affect the performance of the model on other tasks. Smaller-scale models cannot provide even this minimal demonstration, because they lack the variety of tasks necessary to demonstrate robustness. As such, Spaun provides a distinct opportunity to test learning algorithms in a challenging but biologically plausible setting. More generally, Spaun provides an opportunity to test any neural theory that may be affected by being embedded in a complex, dynamical context, reminiscent of a real neural system.

However, Spaun has little to say about how that complex, dynamical system develops from birth. Furthermore, Spaun has many other limitations that distinguish it from developed brains. For one, Spaun is not as adaptive as a real brain, as the model is unable to learn completely new tasks. In addition, both attention and eye position of the model is fixed, making Spaun unable to control its own input. Also, its perceptual and conceptual representations are largely limited to the space of digits from 0 to 9. Anatomically, many

areas of the brain are missing from the model. Those that are included have too few neurons and perform only a subset of functions found in their respective areas. Physiologically, the variability of spiking in the model is not always reflective of the variability observed in real brains (table S3). However, we believe that, as available computational power increases, many of these limitations can be overcome via the same methods as those used to construct Spaun (supplementary section S2.4).

Even in its current form, Spaun offers a distinctly functional view and set of hypotheses regarding the neural mechanisms and organization that may underlie basic cognitive functions. Consequently, Spaun opens new avenues for testing ideas about biological cognition under biologically plausible, more complex, and more functional settings than previously available.

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Supplementary Materials

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Materials and Methods

Supplementary Text

Figs. S1 to S12

Tables S1 to S3

References (21–74)

Movies S1 to S8 (at <http://nengo.ca/build-a-brain/spaunvideos>)

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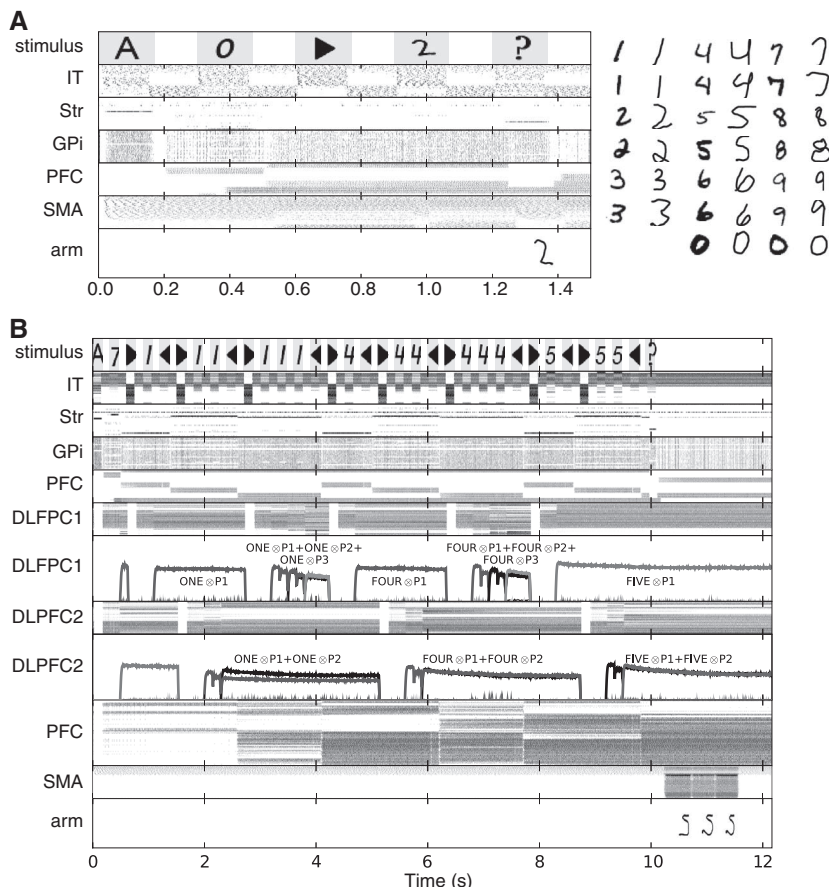


Fig. 3. Time-course plots for two Spaun tasks. **(A)** Results of the copy-drawing task. The input/output pairs for 20 additional runs are shown to the right. **(B)** Results of an example run of the RPM task, plotted using the same method as described in Fig. 2C. See text for details.

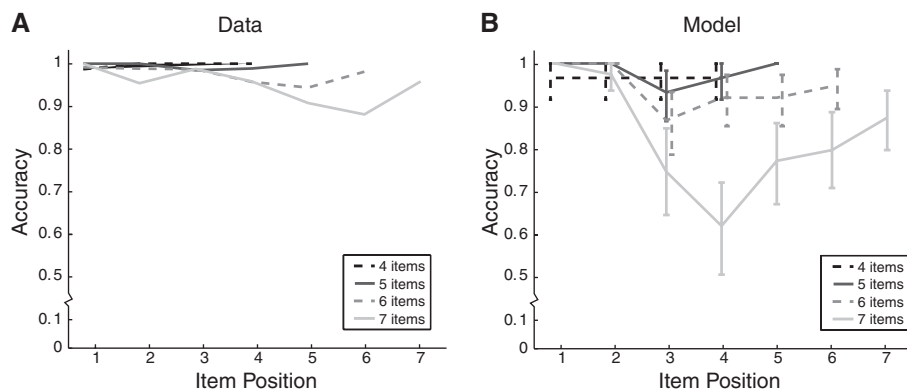


Fig. 4. Population-level behavioral data for the WM task. Accuracy is shown as a function of position and list length for the serial WM task. Error bars are 95% confidence intervals over 40 runs per list length. **(A)** Human data taken from (18) (only means were reported). **(B)** Model data showing similar primacy and recency effects.

Copy Number Variation of Multiple Genes at *Rhg1* Mediates Nematode Resistance in Soybean

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The *rhg1-b* allele of soybean is widely used for resistance against soybean cyst nematode (SCN), the most economically damaging pathogen of soybeans in the United States. Gene silencing showed that genes in a 31-kilobase segment at *rhg1-b*, encoding an amino acid transporter, an α -SNAP protein, and a WI12 (wound-inducible domain) protein, each contribute to resistance. There is one copy of the 31-kilobase segment per haploid genome in susceptible varieties, but 10 tandem copies are present in an *rhg1-b* haplotype. Overexpression of the individual genes in roots was ineffective, but overexpression of the genes together conferred enhanced SCN resistance. Hence, SCN resistance mediated by the soybean quantitative trait locus *Rhg1* is conferred by copy number variation that increases the expression of a set of dissimilar genes in a repeated multigene segment.

Soybean (*Glycine max*) is the world's most widely used legume crop, providing 68% of world protein meal as well as food oil and a renewable source of fuel, with a farm gate value of more than \$35 billion in the United States alone (www.soystats.com). Soybean cyst nematode (SCN; *Heterodera glycines*) is the most economically damaging pathogen of soybean, causing more than \$1 billion in annual losses. SCN has infested most major soybean producing areas worldwide, and there are no practical means of eradication (1).

SCN molts through multiple juvenile and adult life stages, including obligate endoparasitic stages on plant roots, to complete its life cycle (1). Infective J2 juveniles invade roots of both susceptible and resistant soybean hosts, then reprogram host root cells to form feeding sites using highly evolved, secreted nematode effectors (2, 3).

The soybean *Rhg1* (resistance to *H. glycines*) quantitative trait locus on chromosome 18 consistently contributes much more effective SCN resistance than any other known loci (4, 5). *Rhg1* disrupts the formation and/or maintenance of most potential nematode feeding sites (1). Rough-

ly 90% of the commercially cultivated soybean varieties marketed as SCN-resistant in the central United States use the *rhg1-b* allele (haplotype), derived from the soybean line PI 88788, as the main SCN resistance locus. The molecular basis of this SCN resistance has remained unclear.

Genetic mapping has placed *rhg1-b* in an interval that corresponds to a 67-kb segment carrying 11 predicted genes in the genome of the SCN-susceptible but fully sequenced Williams 82 soybean variety (6, 7). It was recently suggested that an amino acid polymorphism in the *Glyma18g02590*-encoded α -SNAP protein in this interval contributes to SCN resistance (8), although the authors indicated that this polymorphism does not account for *rhg1-b*-mediated resistance. None of the gene products within the *rhg1-b* genetic interval resemble canonical plant immune receptors (9).

In the present study, genes from the *rhg1-b* interval (6) were silenced to test for impacts on SCN resistance (10). Transgenic soybean roots expressing artificial microRNA (amiRNA) or hair-

pin (RNA interference) constructs were produced using *Agrobacterium rhizogenes* (11–13). Soybean resistance to SCN was measured 2 weeks after root inoculation by determining the proportion of the total nematode population that had advanced past the J2 stage in each root (Fig. 1A) relative to known resistant and susceptible controls (14). Silencing any one of three closely linked genes at the *rhg1-b* locus of the SCN-resistant soybean variety Fayette significantly reduced SCN resistance (Fig. 1B). Depletion of resistance was dependent on target transcript reduction (fig. S1). Silencing other genes in and around the locus did not affect SCN resistance (e.g., Fig. 1B, genes *Glyma18g02570* and -2620) (10). The three *Rhg1* genes that were found to contribute to SCN resistance encode a predicted amino acid transporter (*Glyma18g02580*), an α -SNAP protein predicted to participate in disassembly of SNARE membrane trafficking complexes (*Glyma18g02590*), and a protein with a WI12 (wound-inducible protein 12) region but no functionally characterized domains (*Glyma18g02610*) (15–17).

Concurrent study of the physical structure of the *rhg1-b* locus revealed an unusual genomic configuration. A 31.2-kb genome segment encoding the above-noted genes is present in multiple copies in SCN resistant lines (Figs. 2 and 3). The DNA sequence of fosmid clone inserts carrying genomic DNA from the *rhg1-b* genetic interval identified a unique DNA junction, not present in the published Williams 82 soybean genome, in which the intergenic sequence downstream of (centromeric to) *Glyma18g02610* is immediately adjacent to a 3' fragment of *Glyma18g02570* (Fig. 2A, fosmids 3, 4, and 5). The genomic repeat contains full copies of *Glyma18g02580*, -2590, -2600, and -2610, as well as the final two exons of *Glyma18g02570*. Whole-genome shotgun sequencing of a line containing *rhg1-b* revealed greater depth of coverage of this interval by a factor of 10 relative to the surrounding chromosomal region or homeologous regions on other chromosomes (Fig. 2B), suggesting the presence of multiple repeats. Further polymerase chain

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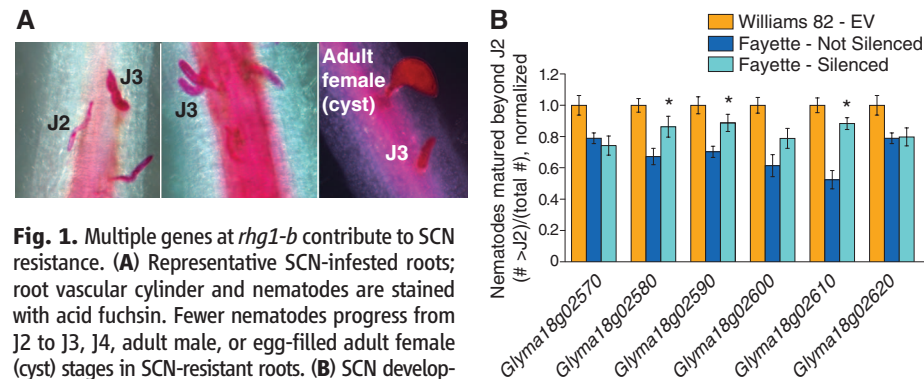


Fig. 1. Multiple genes at *rhg1-b* contribute to SCN resistance. (A) Representative SCN-infested roots; root vascular cylinder and nematodes are stained with acid fuchsin. Fewer nematodes progress from J2 to J3, J4, adult male, or egg-filled adult female (cyst) stages in SCN-resistant roots. (B) SCN development beyond J2 stage in transgenic roots of soybean variety Fayette with the designated gene silenced, relative to Williams 82 (SCN-susceptible) and nonsilenced Fayette (SCN-resistant) controls. Data are means $\pm$ SE. * $P < 0.05$, Fayette (silenced) significantly different from Fayette (not silenced) based on analysis of variance (ANOVA) Tukey test; $P > 0.1$ for *Glyma18g02600*. EV, transformed with empty vector.

reaction (PCR) and sequencing tests confirmed the presence of the *Glyma18g02610-2570* junction in DNA from multiple SCN-resistant soybean accessions, whereas the junction was not detected in four tested SCN-susceptible varieties including Williams 82 (Fig. 2C and fig. S2). The shared identity of the junction sites in disparate sources of SCN resistance suggests a shared origin of the initial resistance-conferring event at *Rhg1*.

Gene expression analysis using quantitative PCR (qPCR) determined that the three genes found to significantly affect SCN resistance show higher transcript levels in roots of SCN-resistant varieties than in susceptible lines (Fig. 2D and fig. S2). This suggested that elevated expression of one or more of the SCN-affecting genes could be a primary cause of elevated SCN resistance. Full-length transcripts were confirmed for *Glyma18g02580*, -2590, and -2610, no transcript was detected for *Glyma18g02600*, and no hybrid repeat-junction transcript was detected for *Glyma18g02570* (fig. S2).

Fiber-FISH (fluorescence in situ hybridization) was used to directly view the arrangement and copy number of the 31-kb repeat segment in different haplotypes of the *Rhg1* locus. The hybridization pattern and DNA fiber length estimates (Fig. 3 and

table S1) indicate a single copy of the repeat in Williams 82, as in the reference soybean genome sequence (7). In Fayette, fiber-FISH revealed 10 copies of the repeat segment per DNA fiber, in the same configuration throughout the multiple nuclei sampled, in a pattern consistent with 10 direct repeats abutting head-to-tail (Fig. 3 and table S1). In samples from Peking, another common source of SCN resistance, three copies per DNA fiber were present in direct repeat orientation (Fig. 3). No additional copies (e.g., at other loci) were evident. *Rhg1* repeat copy number expansion is likely to have occurred by unequal-exchange meiotic recombination events between homologous repeats.

Amino acid polymorphism or overexpression of any one of the three identified *rhg1-b* genes did not account for SCN resistance. From all available *rhg1-b* sequence reads (across multiple repeat copies), no predicted amino acid polymorphisms relative to Williams 82 were identified for *Glyma18g02580*, *Glyma18g02600*, or *Glyma18g02610*. Some copies of *Glyma18g02590* from *rhg1-b* resembled the Williams 82 sequence, whereas others contained a set of polymorphisms, notably at the predicted C-terminal six amino acids of the predicted α -SNAP protein (table S2, confirmed by cDNA sequenc-

ing). However, expression of this non-Williams 82-type *Glyma18g02590* downstream of a strong constitutive promoter or native promoter sequence did not increase the SCN resistance reaction of Williams 82 transgenic roots (Fig. 4 and fig. S3), which suggests that *rhg1-b* SCN resistance requires more than this 2590-amino acid polymorphism. Overexpression of *Glyma18g02580* or *Glyma18g02610* also failed to increase SCN resistance (Fig. 4).

Given the above, simultaneous overexpression of genes within the 31-kb repeat segment was tested as a possible source of SCN resistance. In two separate experiments that together tested >25 independent transgenic events for each DNA construct, resistance to SCN was significantly increased in SCN-susceptible Williams 82 by simultaneous overexpression of the set of genes (Fig. 4; see also fig. S4A). A DNA construct overexpressing *Glyma18g02580*, -2590, and -2610 (but not -2600) also conferred enhanced SCN resistance (fig. S4B). The collective findings indicate that *Rhg1*-mediated SCN resistance is attributable to elevated expression of *Glyma18g02580*, -2590, and -2610.

These results reveal a novel mechanism for disease resistance: an expression polymorphism

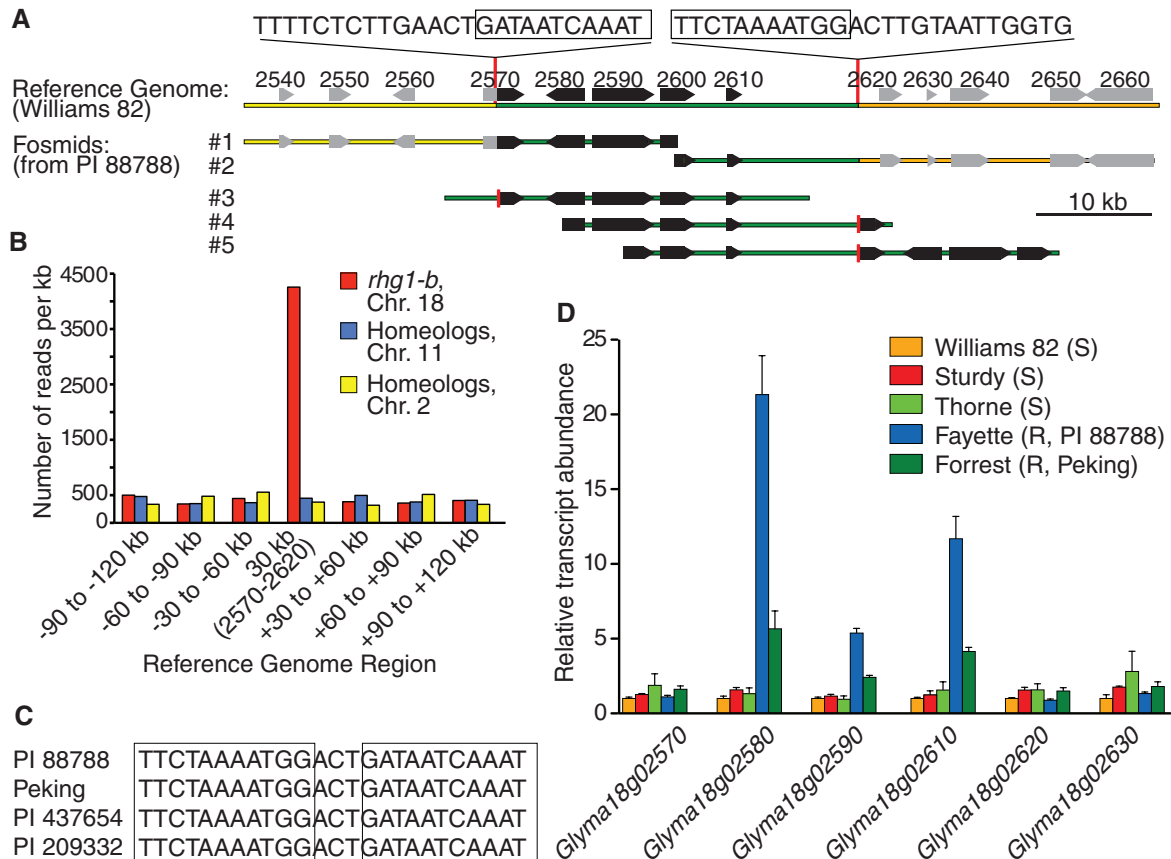


Fig. 2. A 31.2-kb repeat that elevates expression of the encoded genes is present in SCN-resistant haplotypes of the *Rhg1* locus. (A) Diagram of *Rhg1* locus of Williams 82 (top) and five fosmid inserts from *rhg1-b* haplotype. Numbers and block icons refer to soybean genes (e.g., *Glyma18g02540*). Fosmids 3, 4, and 5 carry *rhg1-b* genome segments that span repeat junctions. (B) Number of whole-genome shotgun sequencing reads corresponding to *Rhg1* [green region shown in

(A)] was greater by a factor of 10 than for adjacent genomic regions on chromosome 18 or for homeologous regions. (C) Sequence of unique *Rhg1* repeat junction not found in reference genome, from four different sources of SCN resistance. (D) Transcript abundance of genes encoded in 31-kb repeat region is greater in roots from SCN-resistant varieties than from SCN-susceptible varieties. Means $\pm$ SE shown for qPCR; results for *Glyma18g02600* were at limit of detection.

Fig. 3. Fiber-FISH detection of *Rhg1* copy number variation in widely used soybean lines. (A) Two adjacent probes were isolated from a single PI88788 (*rhg1-b*) genomic DNA fosmid clone whose insert spans a repeat junction, generating the 25.2-kb (green label) and 9.7-kb (red label) probes that correspond to the Williams 82 chromosome 18 sequence as shown. (B) Probe diagram and composite of four Fiber-FISH images (four DNA fibers) per genotype, revealing 10 or 3 direct repeat copies of the 31-kb *Rhg1* segment in SCN-resistant Fayette and Peking and one copy per *Rhg1* haplotype in SCN-susceptible Williams 82. White bars = 10 μm , which correspond to approximately 32 kb using a 3.21 kb/ μm conversion rate (32).

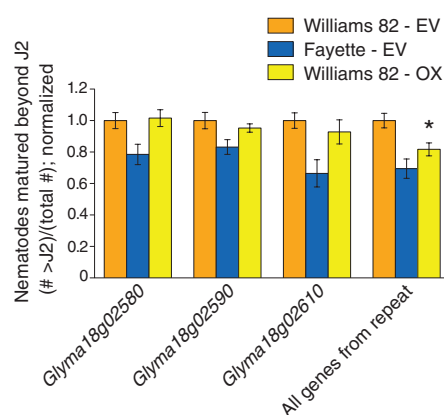
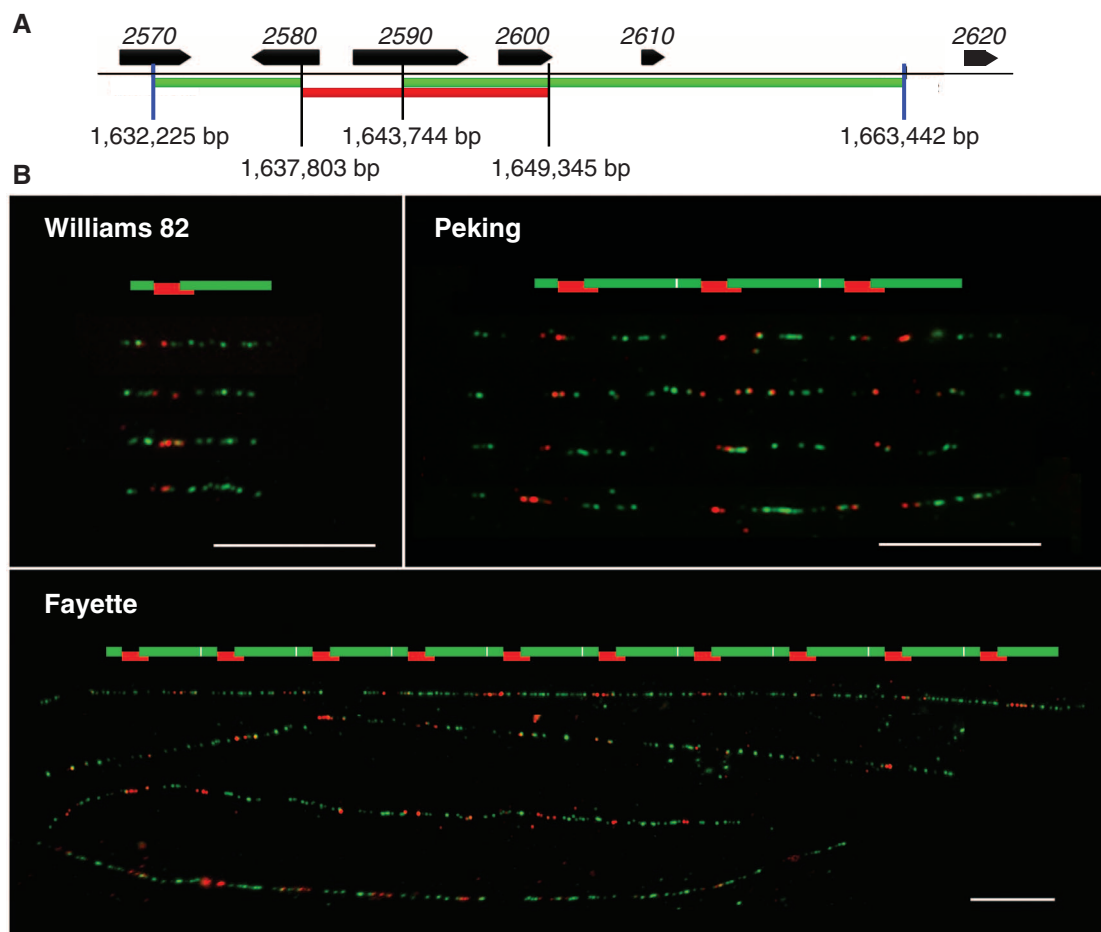


Fig. 4. Elevated SCN resistance conferred by simultaneous overexpression of multiple genes rather than overexpression of individual genes from the 31-kb *rhg1-b* repeat. SCN development beyond J2 stage is reported for transgenic soybean roots (variety Williams 82) overexpressing the designated single genes, or overexpressing all genes encoded within the 31-kb repeat (*Glyma18g02580*, -2590, -2600, and -2610), relative to Williams 82 (SCN-susceptible) and Fayette (SCN-resistant) controls. Data are means $\pm$ SE for roots transformed with empty vector (EV) or gene overexpression constructs (OX). * $P < 0.05$, Williams 82 with gene construct significantly different from Williams 82 EV based on ANOVA Tukey test.

for multiple disparate but tightly linked genes, derived through copy number variation at the *Rhg1* locus. This suggests future approaches to enhance *Rhg1*-mediated quantitative resistance against the globally important SCN disease of soybean—for example, through isolation of soybean lines that carry more copies of the 31-kb *Rhg1* repeat. Transgenic overexpression of the native or altered genes may improve SCN resistance and/or be applicable in other species for resistance to other endoparasitic nematodes.

The biochemical mechanisms of *Rhg1*-mediated resistance remain unknown. Other sequenced plant genomes do not carry close homologs of the predicted Glyma18g02610 protein, although a wound-inducible protein in ice plant with 55% identity has been studied (17). Modeling of the Glyma18g02610 predicted tertiary structure suggested (10) that Glyma18g02610 may participate in the production of phenazine-like compounds that are toxic to nematodes. The Glyma18g02590 α -SNAP protein is likely involved in vesicle trafficking and may influence exocytosis of products that alter feeding site development or nematode physiology (18). Because it is one of at least five α -SNAP homologs encoded in the soybean reference genome, Glyma18g02590 may have undergone subfunctionalization or neofunctionalization (19). The Glyma18g02580 protein and its most closely re-

lated plant transporter proteins are not functionally well characterized, but Glyma18g02580 contains a predicted tryptophan/tyrosine permease family domain. Tryptophan shares structural similarity with and is a precursor of the auxin hormone indole-3-acetic acid, which suggests the intriguing possibility that Glyma18g02580 may affect functionally important auxin levels or distribution (2, 3). Together, these genes create an unfavorable environment at nematode feeding sites.

Growing evidence from metazoa and plants suggests that genome structural variation is a frequent and powerful driver of phenotypic diversity (20, 21). Copy number variation of chromosomal subsegments (beyond simple duplication) can affect gene expression levels (22), and single-gene copy number variation contributes to a number of adaptive traits in humans, plants, and insects (23–26). Recent analyses of genome architecture in sorghum, rice, and soybean have reported high levels of copy number variation and a tendency for these genomic regions to overlap with postulated biotic and abiotic stress-related genes (27–29).

Our work provides a concrete example of copy number variation in which the repeat encodes multiple gene products that contribute to a valuable disease resistance trait. Single-copy clusters of genes that are functionally related but non-homologous are highly unusual in multicellular

eukaryotes, but such clusters have been reported in association with plant secondary metabolism (30, 31). Given the repetitive and plastic nature of plant genomes and the relatively underexplored association between copy number variation and phenotypes, it seems likely that a number of other complex traits are controlled by this type of structural variation.

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Supplementary Materials

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Materials and Methods
Figs. S1 to S4
Tables S1 to S3
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An Exon Splice Enhancer Primes IGF2:IGF2R Binding Site Structure and Function Evolution

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Placental development and genomic imprinting coevolved with parental conflict over resource distribution to mammalian offspring. The imprinted genes *IGF2* and *IGF2R* code for the growth promoter insulin-like growth factor 2 (IGF2) and its inhibitor, mannose 6-phosphate (M6P)/IGF2 receptor (IGF2R), respectively. M6P/IGF2R of birds and fish do not recognize IGF2. In monotremes, which lack imprinting, IGF2 specifically bound M6P/IGF2R via a hydrophobic CD loop. We show that the DNA coding the CD loop in monotremes functions as an exon splice enhancer (ESE) and that structural evolution of binding site loops (AB, HI, FG) improved therian IGF2 affinity. We propose that ESE evolution led to the fortuitous acquisition of IGF2 binding by M6P/IGF2R that drew *IGF2R* into parental conflict; subsequent imprinting may then have accelerated affinity maturation.

The sequence of molecular evolutionary events that established placental viviparity, genomic imprinting, and parental conflict in mammals remain poorly understood (1). Genomic imprinting occurs when expression of one allele of a diploid gene is silenced depending on the parent of origin, either from the father or the mother. Parental conflict over the distribution of resources to offspring has been supported by the observation of reciprocal imprinting of genes coding for the growth promoter insulin-like growth factor 2 (IGF2) and the cation-independent mannose 6-phosphate/IGF2 receptor (M6P/IGF2R or IGF2R) (2). *IGF2* and *IGF2R* are 2 of the ~80 genes imprinted in mammals and two of the five genes (with *INS*, *MEST/PEG1*, and *PEG10*) imprinted in marsupials. So far, no

evidence supports the existence of imprinting in monotremes despite the presence of a yolk sac placenta (3, 4). On the basis of functional data, IGF2R transports M6P-modified acid hydrolases to pre-lysosomes (5). Of the 15 extracellular domains of IGF2R, domain 11 binds IGF2 in therians and internalizes the ligand for degradation, whereas M6P ligands bind to domains 3, 5, and 9 (5). *Igf2* rescues placental-dependent embryonic lethality associated with laboratory-created murine parthenogenesis, implicating IGF2 supply as a regulator of placental development (6). Disruption of the maternal *Igf2r* allele results in *Igf2*-dependent overgrowth and fatality, supporting that IGF2R antagonizes the function of IGF2 (7, 8). The structure of the unbound human domain 11 shows that the IGF2 binding

site is composed of defined loops (AB, CD, FG, and HI; Fig. 1A and fig. S1) but how this domain 11 evolved to bind IGF2 and the relation to imprinting coevolution remains unknown (9–12).

We established a high-resolution structure of the human IGF2R:IGF2 complex and then compared this to other phylogenetically informative vertebrates. We adopted a nuclear magnetic resonance (NMR) approach because the side chain amino acid interactions across the binding interface were not resolved in our 4.1 Å resolution cocrystal structures (9). Wild-type (WT) human domain 11 and IGF2 failed to form a stable association in initial NMR studies. However, we identified an AB loop mutant (domain 11^{E1544K, K1545S, L1547V} or clone E4¹) with an increased affinity for IGF2 that formed a tight complex [binding affinity (K_D) = 15 nM versus 46 to 64 nM for WT domain 11; Table 1 and ¹H-¹⁵N correlation spectra, figs. S2 and S3] (12). We solved the solution structure of this 24.2-kD complex (IGF2: domain 11^{E4}) with NMR structural and quality statistics in table S1. When free IGF2 (Fig. 1A) binds to the single domain 11^{E4}

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(Fig. 1B), ~640 to 760 Å² of solvent-accessible surface area is buried on domain 11^{E4} and ~600 to 820 Å² on IGF2 (Fig. 1C), similar to the crystal structure of IGF2 in complex (710 to 750 Å²) (9). Domain 11^{E4} retains a relatively fixed conformation of the CD loop upon complex formation (Fig. 1, C and D). The mutated IGF2R AB loop also moves to accommodate IGF2 helix 1 and the packing of IGF2 residues T16 and F19, whereas the IGF2R FG loop is repositioned between helices 2 and 3 of IGF2 and accommodates burial of IGF2 residue L53 in the domain 11^{E4} binding site. All three of these IGF2 residues are critical for IGF2R binding (13). Both conformational changes allow the formation of complementary hydrophobic surfaces and correctly support a range of H-bonding and salt-bridging interactions (Fig. 1, D, E, and F; fig. S4; movie S1) (14). Three IGF2R domain 11 residues

[V1574, L1626, and L1636 (15)] form a foundation for the three pronged interactions of IGF2 with key domain 11 residues: namely hydrophobic residues IGF2 F19 with domain 11 F1567, L1629, and Y1542 and IGF2 L53 with domain 11 K1631, with a third interaction where IGF2 specificity is conferred by T16 interactions with domain 11 Y1606 and I1572 (Fig. 1).

We expressed and performed binding studies of recombinant domain 11-His₆ and NusA-IGF2 fusion proteins from different species in the yeast *Pichia pastoris* and bacteria *Escherichia coli*, respectively (figs. S5 to S7). IGF2 from each species bound human IGFBP-3 with similar nanomolar affinity (figs. S7 and S8). Initial analysis of species specific domain 11 binding to NusA-IGF2 fusion protein revealed a *K_D* of human and opossum between 100 and 130 nM; platypus and echidna revealed a lower affinity

interaction, with a *K_D* range between 300 and 900 nM (fig. S6). We confirmed the absence of binding of purified IGF2 to IGF2R domain 11 in chicken and zebrafish and the lower IGF2 binding in opossum compared with human (Table 1 and fig. S9) (16, 17). Purified echidna IGF2 (7866.8 dalton) bound human IGF1R, IGFBP-2, and IGFBP-3 and purified echidna domain 11 with affinities similar to that of human IGF2 (14) (figs. S8 and S9). We confirmed that purified echidna IGF2 binds echidna domain 11 with a steady-state *K_D* of 385 ± 13 nM (standard deviation, Table 1). These data contradict a previous report where non-recombinant platypus IGF2R failed to bind human IGF2 at a fixed 2 nM concentration, a concentration that our data suggest may be too low for binding detection (3).

IGF2 is highly conserved across all vertebrate species including IGF2R-interacting residues

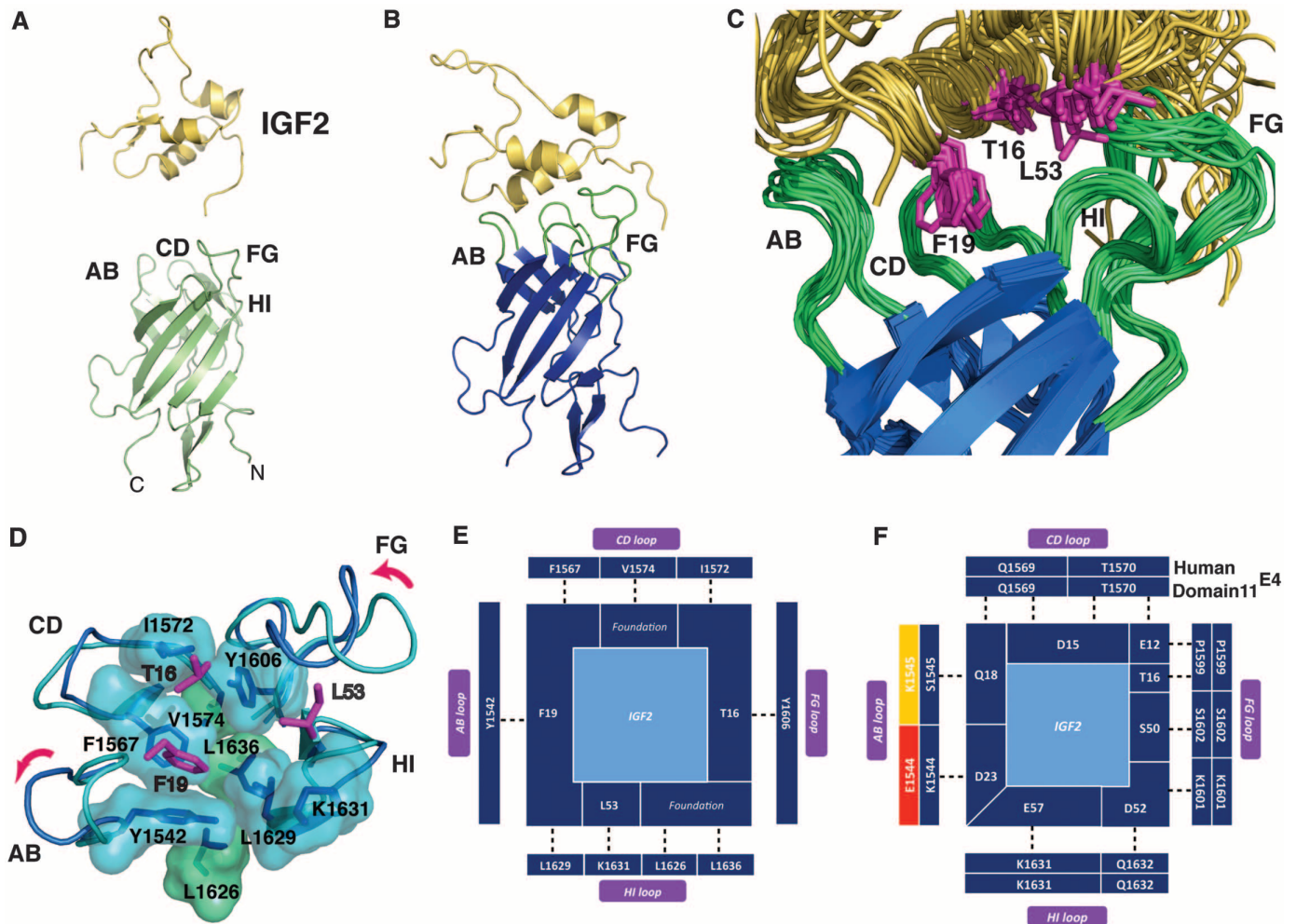


Fig. 1. (A) Solution structure of free IGF2 (yellow), the free domain 11^{E4} (light green), the characteristic domain 11 β barrel, and four loops involved in IGF2 binding (AB, CD, FG, HI). (B) The 24.2-kD complex of domain 11^{E4} (blue) bound to IGF2 (yellow) solved by NMR. (C) Superimposition of an ensemble of 20 domain 11^{E4} low-energy NMR structures showing the IGF2 binding pocket. The AB, CD, FG, and HI loops are shown in green. (D) Backbone and surface representation of the IGF2 binding pocket highlighting a group of nine hydrophobes on domain 11^{E4}, including the three foundation residues (L1626, L1636, and V1574) (15) that support the binding pocket (green) and, in light blue, the hydrophobes that

form the IGF2 binding pocket. The flexible AB and FG loops change conformation upon complex formation (purple arrows). (E) Hydrophobic binding residues on IGF2 (center) and binding partners (dark blue, indicating favorable hydrophobic interactions) on domain 11^{E4} over the AB, CD, FG, and HI loops (clockwise). (F) The nonhydrophobic groups (charged, polar, and hydrogen bond interactions) of IGF2 that interact with domain 11^{E4} are shown with matching complementarity in dark blue. Incorrect charge and polar complementarity are shown in red. Yellow represents where either the acquisition of a charge or change in steric bulk of a residue cannot be assessed in the absence of a high-resolution structure.

T16, F19, and L53 (fig. S1) (15). Evolution of the IGF2:IGF2R interaction was likely to be dependent on the changes to the binding pocket of domain 11. We solved the solution NMR structures of domain 11 from opossum, echidna, and chicken (table S2). These structures all adopt the same topology as human domain 11, with a flattened β barrel (9) (Fig. 2A). Differences between the structures were observed in the flexible loops and foundation regions, in particular those comprising the IGF2 binding site (Fig. 2B). As domain 11 evolved from a non-IGF2 binding domain (chicken) to acquire IGF2 binding (echidna) and then to higher affinity binding (opossum, human), the surface surrounding the IGF2 binding region displays a loss of charged residues, increased volume and hydrophobicity, and assembly of the correct shape complementarity (Fig. 2, C and D) (14). The charge distribution, primarily located in the AB and CD loops, changed from an overall negative potential to a positive potential to complement the negative electrostatic surface of IGF2 (Fig. 2D and fig. S10). The maps show the gradual accumulation of optimal residue interactions with IGF2 in all the loops, including the foundation residues, suggesting evolutionary maturation of the binding site topology and affinity (Fig. 2, C and D). We then replaced the longer nonbinding chicken CD loop (amino acids 1144 to

1154) with the shorter echidna CD loop and rescued similar binding kinetics to the intact echidna domain, demonstrating the central importance of the CD loop in IGF2 binding (Table 1 and fig. S11). The echidna CD loop–driven binding site acquisition in chicken was augmented by the addition of echidna AB and FG loops that stabilized the interaction (table S3). As expected, mutations of hydrophobic residues of the shortened echidna CD loop in chicken abolished binding, as did other amino acids [E1568→D1568 (E1568D), G1571K, and T1570P (15)], underlining the importance of a rigid structural topology of the CD loop for IGF2 binding (table S3).
A domain 11 hydrophobic pocket, although critical for IGF2 binding, is not the only factor necessary for a high-affinity interaction between these proteins (9). Some residues in the AB loop of echidna domain 11 are more similar to non-mammalian sequences than to the therian domain 11 and may result in a lower binding affinity (14). Human IGF2R domain 13 acts through a fibronectin type II domain–like insert to stabilize the AB loop of domain 11, an effect that enhances affinity that we also detected for echidna IGF2R domains 11 to 13 compared with the single domain (9) (fig. S11).
Analysis of exon 33 and 34 DNA sequences that code for domain 11 predicted a dense exon

splicing enhancer (ESE) cluster within exon 34 in platypus, a region that only codes for the CD loop of domain 11 (Fig. 3A and fig. S12) (14). A shift in the dependency from intron- to exon-based gene splicing occurred during evolution to multicellular eukaryotes (18), presumably in order to improve efficient generation of mRNA without extended introns. In monotremes, ESEs may have been important because of intronic expansion resulting from insertions of multiple repeat elements (19). We tested whether the ESE in the 5' region of exon 34 was functional by using *in vivo* splicing assays of minigenes from the chicken and platypus CD loop sequences (Fig. 3, B and C). Splicing of intron 33 containing minigenes in chicken DF-1 cells identified a cryptic splice (CS) acceptor site that caused a frame shift and premature stop codon in chicken exon 34 (Fig. 3B and fig. S13). In this system, replacing the chicken exon 34 ESE sequence with that of the same region of platypus suppressed the appearance of the CS product, suggesting that the platypus CD loop sequence contained a functional ESE (Fig. 3B). We next tested the CD loop sequences *in vitro* with an ESE-dependent *doublesex* (*dss*) minigene splicing system compared to a positive control (AAG)₇ (14) (Fig. 3C and fig. S14). In agreement with ESE predictions, platypus and human ESEs promoted rapid accumulation of spliced product quantified by using reverse transcription

Table 1. Species-specific real-time binding analysis of recombinant IGF2R domain 11 interactions with IGF2. k_a^1 indicates association (on) rate; k_d^1 , dissociation (off) rate; RU, response units. Binding to IGF2 assessed with BIAcore T200 using purified biotinylated IGF2 on a streptavidin biosensor chip. Echidna IGF2 is (peak 2) purified recombinant IGF2 (fig. S8). Recombinant proteins expressed in *E. coli* and, for opossum, chicken, and zebrafish domain 11 and chicken with echidna

CD-loop binding to IGF2, *P. pastoris*. All experiments repeated at least four times except human domain 11–chicken IGF2 and echidna domain 11–13–human IGF2 (repeated twice) and human IGF2 binding to human domain 11^{E4}, chicken with echidna CD loop, and echidna domain 11–13 (on two independent BIAcore chips). BIAcore 3000 chip used for echidna domain 11–13–human IGF2 binding. Dash entry means no binding; ND, not determined.

	Major binding kinetic variables (two-state conformational change model)				Steady-state binding kinetics	
	k_a^1 ($\times 10^5 \text{ m}^{-1} \text{ s}^{-1}$) mean	k_d^1 ($\times 10^{-2} \text{ s}^{-1}$) mean	K_D ($\times 10^{-9} \text{ M}$) mean $\pm$ SD	χ^2 (RU ²) mean	K_D ($\times 10^{-9} \text{ M}$) mean $\pm$ SD	χ^2 (RU ²) mean
Human domain 11 ^{E4} binding to IGF2						
Human IGF2	27.6	4.4	15.3 $\pm$ 2.0	0.41	15.5 $\pm$ 3.3	0.77
Human domain 11 binding to IGF2						
Human IGF2	50	23	46 $\pm$ 1.8	0.33	64 $\pm$ 1.0	0.24
Echidna IGF2	20	10.6	53 $\pm$ 2.7	0.25	77 $\pm$ 0.3	0.1
Chicken IGF2	23	12	50 $\pm$ 4.0	1.90	63 $\pm$ 1.7	1.0
Opossum domain 11 binding to IGF2						
Human IGF2	6.9	10.8	130 $\pm$ 8.8	0.59	164 $\pm$ 1.0	0.86
Echidna IGF2	6.5	10.6	135 $\pm$ 0.5	0.56	157 $\pm$ 2.5	0.70
Chicken IGF2	17.6	35.0	171 $\pm$ 2.2	0.10	219 $\pm$ 6.6	0.08
Echidna domain 11 binding to IGF2						
Human IGF2	3.8	10.6	244 $\pm$ 2.0	0.08	329 $\pm$ 10	0.23
Echidna IGF2	4.5	13.8	256 $\pm$ 2.0	0.54	385 $\pm$ 13	0.14
Chicken IGF2	9.2	25.9	244 $\pm$ 2.0	0.08	412 $\pm$ 10	0.03
Chicken and zebrafish domain 11 binding to IGF2						
Human IGF2	–	–	–	–	–	–
Echidna IGF2	–	–	–	–	–	–
Chicken IGF2	–	–	–	–	–	–
Chicken with echidna CD loop binding to IGF2						
Human IGF2	1.0	4.1	434 $\pm$ 1.0	2.0	325 $\pm$ 10	0.20
Echidna domain 11–13 binding to IGF2						
Human IGF2	2.6	3.2	120 $\pm$ 10	1.9	ND	ND

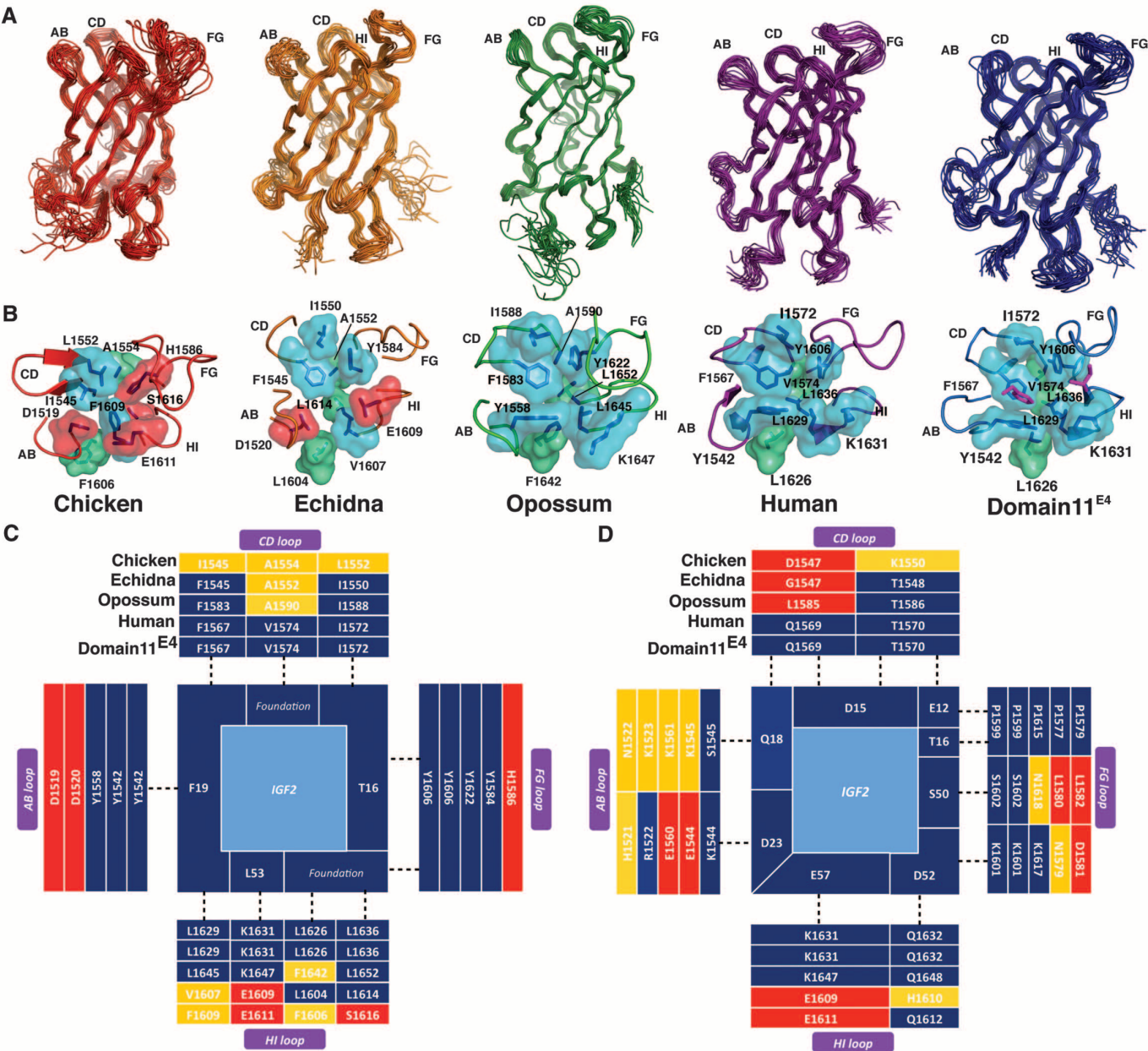


Fig. 2. (A) High-resolution NMR structures of domain 11 from chicken (red), echidna (orange), opossum (green), and human (magenta, PDB:2CNJ) and domain 11^{E4} (blue). (table S2 provides a summary of structural statistics.) Ensembles of the lowest 20 energy models are shown for each species. **(B)** Surface representations of the binding pocket of IGF2R–domain 11 and the acquisition of

an increased hydrophobicity surrounding the IGF2 binding pocket (movie S1) (15). **(C)** Hydrophobic binding residues on IGF2 (center) and binding partners (dark blue) on domain 11^{E4}, human, opossum, echidna, and chicken over the AB, CD, FG, and HI loops. **(D)** Evolution of favorable charged, polar, and hydrogen bond interactions between IGF2 and domain 11 species. Colors as in Fig. 1, E and F.

polymerase chain reaction (RT-PCR) and capillary electrophoresis.

In comparisons of ESE densities of all 6144 possible combinations of codons for the platypus CD loop, we found 6124 of the 6144 sequences, that is, >99.67%, to have a lower ESE density than the sequence present in platypus, with only 10 codons containing more ESEs (fig. S12). Compared with platypus, the human CD loop DNA sequence exhibited relatively reduced ESE density but showed a higher degree of codon adaptation for the coded amino acids (14). Although it is possible that the ESE arose after the CD loop–

dependent binding site was established, these data suggest that a functional ESE in exon 34 changed the amino acid sequence of the CD loop and thereby lead to the fortuitous creation of the IGF2 binding site in a M6P receptor. The evolution of splicing is underpinned by a complicated multifactorial network of molecular interactions, which cannot be expected to reduce to a single factor (20). We speculate that the observed monotreme ESE clustering may have occurred in a common mammalian ancestor, perhaps as a response to intron expansion, in agreement with genome-wide correlations (21). The subsequent reduction

of ESE density observed in humans may have been due to further selective pressure to improve IGF2 binding.

Our data suggest that the IGF2 binding site on IGF2R was induced through evolution of an ESE specifically coding for the CD loop, and the CD loop alone. Because the structure and function of the CD loop is conserved in therians, we propose that binding initially occurred in a common ancestor of monotremes, marsupials, and eutherian mammals. The absence of *IGF2R* imprinting in monotremes (3, 22) is supported by a therian-specific mechanism of silencing of transposons

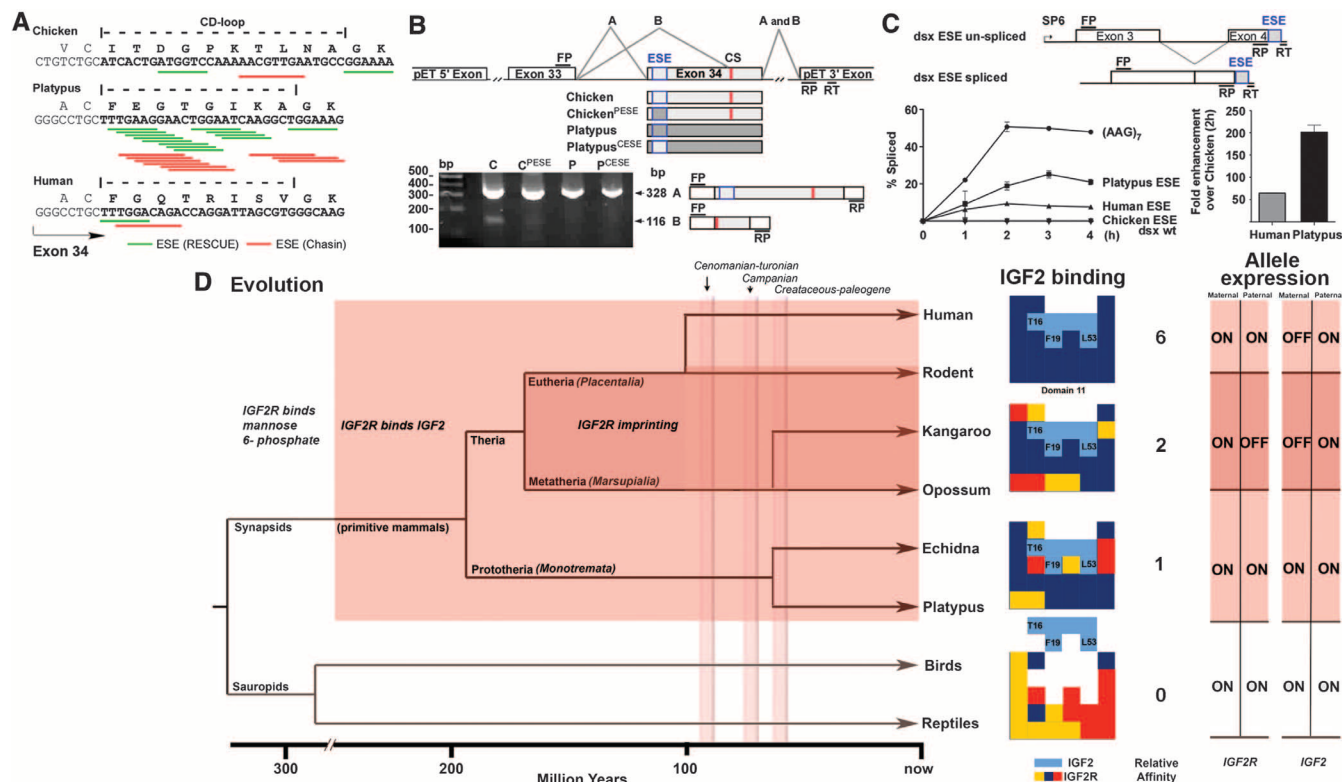


Fig. 3. (A) ESE densities at the CD-loop coding region of exon 34. The positions of predicted hexamer (Rescue-ESE) and octamer (Chasin) ESEs are illustrated (fig. S12). (B) In vivo splicing of chicken, platypus, or hybrid exons 34 in chicken DF-1 cells (sequences of splice products provided in fig. S13). Two complete splice products, A and B, (cryptic splice site, CS) are shown, with RT-PCR gel products showing expression of A product and suppression of B product by ESEs. FP, RP, and RT are forward, reverse, and reverse transcriptase primers, respectively. (C) Minigene constructs and comparative enhancement of dsx minigene splicing in HeLa cell nuclear extracts by control (AAG₇ repeat) and ESEs (fig. S14). (D) Phylogenetic context implies that IGF2-IGF2R binding site acquisition (light shade)

occurred before the appearance of imprinting (dark shade) but was present within prototheria. Relative affinity increased in methatheria compared with prototheria, in keeping with a transition in binding site structure (CD loop). *IGF2R* is bi-allelically expressed in human, presumably because less selection pressure exists to maintain monoallelic expression, and limits *IGF2R* imprinting to nonprimate eutherians and metatheria. In terms of binding, favorable protein interactions are shown in blue, with incorrect charge and unpredictable complementarity shown in red and yellow, respectively. Silenced (imprinted) *IGF2R* allele is shown as OFF compared with the expressed allele as ON. For *IGF2*, the reciprocal imprinted alleles are present in both methatheria and eutheria but absent in prototheria.

(long terminal repeat) by methylation (23) and by lack of germline restriction of BORIS expression (24). These data suggest that the acquisition of the IGF2 binding site occurred before *IGF2R* imprinting. We speculate that once the binding interaction with IGF2R was established, the conserved transportation function of IGF2R as a M6P receptor mediated a reduction of bioavailable IGF2. The subsequent increase in IGF2 affinity was selected for with respect to, -AB, -FG, and -HI (non-CD) loop changes, coincident with the onset of imprinting and consistent with parental conflict (25). Affinity maturation through purifying selection for improved regulation of IGF2 supply may have been accelerated by imprinting induced monoallelic IGF2R expression (26).

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Supplementary Materials

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Cytoplasmic ATP Hydrolysis Powers Transport of Lipopolysaccharide Across the Periplasm in *E. coli*

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Millions of molecules of lipopolysaccharide (LPS) must be assembled on the *Escherichia coli* cell surface each time the cell divides. The biogenesis of LPS requires seven essential lipopolysaccharide transport (Lpt) proteins to move LPS from the inner membrane through the periplasm to the cell surface. However, no intermediate transport states have been observed. We developed methods to observe intermediate LPS molecules bound to Lpt proteins in the process of being transported in vivo. Movement of individual LPS molecules along these binding sites required multiple rounds of adenosine triphosphate (ATP) hydrolysis in vitro, which suggests that ATP is used to push a continuous stream of LPS through a transenvelope bridge in discrete steps against a concentration gradient.

The outer membrane of Gram-negative bacteria is an asymmetric bilayer, with the inner leaflet composed of phospholipids and the outer leaflet composed of lipopolysaccharide (LPS). The LPS layer allows Gram-negative bacteria to survive in harsh environments and in the presence of many antibiotics. However, phospholipids and LPS do not spontaneously assemble into an asymmetric bilayer, and the cell devotes substantial resources to synthesizing LPS at the inner membrane and assembling it in the outer membrane (1). The process by which hydrophobic LPS molecules are transported across two membranes and the intervening aqueous periplasmic space is not understood, but it is known to be mediated by seven essential Lpt (lipopolysaccharide transport) proteins. The heteromeric ATP-binding cassette (ABC) transporter LptBFG forms a complex in the inner membrane with a membrane-bound protein, LptC (Fig. 1A) (2–5). Using homologous domains, the C terminus of LptC interacts with the N terminus of LptA, and the C terminus of LptA interacts with the N-terminal periplasmic domain of LptD (6–9). The resulting head-to-tail oligomer produced creates a transenvelope protein bridge that connects the inner-membrane Lpt components with the outer-membrane LPS translocon (LptD and LptE) (6, 10–15). Defects in any of the Lpt proteins cause LPS to accumulate in the outer leaflet of the inner membrane (2, 16). However, the mechanism of LPS transport has been difficult to study because it involves two membranes and seemingly cannot be broken into different steps.

To elucidate the mechanism of LPS transport, we first sought to identify sites where

LPS interacts with the Lpt proteins in vivo. We incorporated an unnatural amino acid containing a photo-cross-linker (*p*-benzoylphenylalanine, *p*BPA) at 23 positions in LptC and 14 positions in LptA (17–20). Four of the LptC mutants and five of the LptA mutants formed cross-links to LPS in an ultraviolet light (UV)-dependent manner (Fig. 1, B and C). LptC adducts contained the cross-linker at positions 47, 78, 172, and 182 (Fig. 1B), whereas LptA adducts contained the cross-linker at positions 32, 36, 95, 114, and 116 (Fig. 1C). Except for residue 47 in LptC, all of these LPS adducts formed on the inside of the β -jellyroll structures. Residue 47 in LptC is in a disordered region of the protein (9), which may become ordered when it interacts with the LptBFG complex or LPS. No cross-links from side chains on the outer surface of the β jellyroll of LptC or LptA were observed. Thus, we propose that LPS specifically binds inside these proteins and transits the periplasm on the inside of the β jellyroll of these proteins.

To determine whether LptC or LptA alone can extract LPS from the inner membrane or whether cross-linking to LPS is dependent on the entire transenvelope complex, we manipulated the expression levels of the Lpt components. We overexpressed LptC or LptA alone or in combination with the other inner-membrane Lpt proteins (i.e., LptBFG or LptBFGC), reasoning that LptC and LptA would mostly not reside in Lpt complexes if they were overexpressed individually. Cross-linking of LPS to LptC at position 47, but not at positions 78, 172, or 182, increased substantially when LptBFG was co-overexpressed (Fig. 2A). Thus, when LptA, LptD, and LptE are limiting, LPS accumulates near residue 47 of LptC. Position 47 may be located near a strong binding site for LPS, whereas the other cross-linking positions may reflect more transient interactions that occur during the movement of LPS through fully assembled bridges. Because LPS accumula-

tion at the binding site around residue 47 depends on LptBFG, it is also clear that LptC cannot extract LPS directly from the inner membrane unaided.

All five LptA mutants showed increased cross-linking with LPS when they were co-overexpressed with LptBFGC (Fig. 2B). Thus, LptA cannot receive LPS directly from the inner membrane, but requires LptBFGC complex. LPS must migrate out of the inner membrane in an ordered process that requires LptBFG to get to LptC and LptBFGC to get to LptA. Because LPS transport between these proteins can occur in incomplete Lpt bridges, it was possible to develop a biochemical system to separate the individual step of LPS release from the membrane from that of the transport step along the Lpt bridge.

To study the ATP dependence of LPS extraction from the inner membrane, we first prepared spheroplasts containing overexpressed LptC(T47*p*BPA) and LptBFG by disrupting the outer membrane and cell wall with ethylenediaminetetraacetic acid (EDTA) and lysozyme. The inner membrane was then permeabilized by osmotic shock to introduce buffer with or without ATP into the cytoplasm to generate right-side-out (RSO) membrane vesicles (21–25). These vesicles were then irradiated with UV light. Substantial LPS cross-linking to residue 47 of LptC was only observed when LptBFG was co-overexpressed and ATP was present (Fig. 3A). A small amount of LPS was cross-linked to LptC in the absence of ATP, but this amount did not increase over time (Fig. 3A). Presumably this small amount of LPS was present before generating the RSO fraction. Because cross-linking increased as a function of time in the presence of ATP, transfer of LPS from the inner membrane to the membrane-bound LptC appears to require energy.

We next addressed whether LPS could be transferred to the periplasmic component, LptA, in this in vitro system. This reconstitution requires release of LPS from a membrane fraction to a soluble periplasmic protein (fig. S1). Purified LptA(I36*p*BPA), which cross-linked LPS in vivo, was added to RSO vesicles and analyzed for cross-linking to LPS. LPS cross-linked to LptA over time only in the presence of ATP and when all four inner-membrane components were overexpressed (Fig. 3B). Notably, LPS did not cross-link to LptA in the absence of LptC overexpression; this finding indicates that LPS must first be transferred to LptC before being transferred to LptA, which is consistent with previous work (9).

To confirm that the soluble periplasmic bridge component assembled in this in vitro system reflects the in vivo assembly process, we tested the specificity of this cross-linking. An LptA mutant, LptA(H37*p*BPA), interacts directly with LptC (6) but not with LPS in vivo (Fig. 1C). Residues Ile³⁶ and His³⁷ are both located on the edge of the β jellyroll that interacts with LptC,

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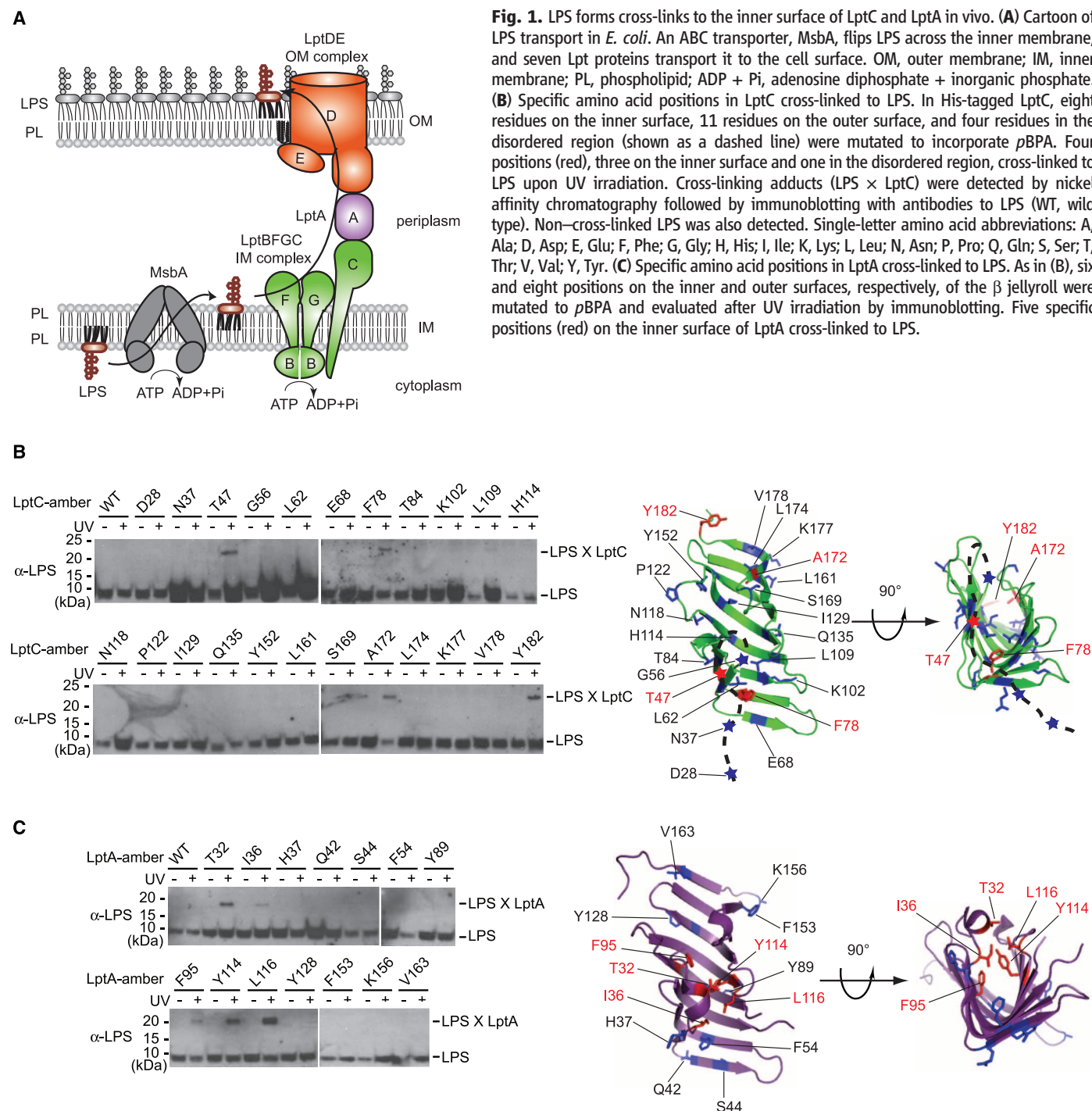
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but their side chains are oriented in opposite directions (Fig. 3C). In this RSO vesicle reconstitution, the His³⁷ mutant cross-linked to LptC (Fig. 3D). However, although His³⁷ is directly adjacent to Ile³⁶, His³⁷ did not cross-link to LPS in the presence of ATP (Fig. 3B). Thus, our *in vitro* reconstitution cleanly recapitulates the specific protein-protein and the protein-substrate interactions observed *in vivo*. Because the binding of LptA to LptC was rapid and did not depend on time or the presence of ATP (Fig. 3D), energy appears not to be required for assembly of the

bridge, but is required to transport LPS from the inner membrane to LptA.

A small amount of LPS accumulated in LptC in the absence of ATP (Fig. 3A) but did not spontaneously move to LptA (Fig. 3B). We thus asked whether LPS transfer from LptC to LptA requires a separate energetic step. We incubated RSO vesicles overexpressing LptBFGC-LptC(T47pBPA) with ATP to accumulate LPS in LptC, and then added purified LptA(I36pBPA) with or without an adenosine triphosphatase (ATPase) inhibitor, vanadate, before irradiating the samples.

LPS did not cross-link to LptA in the inhibitor-treated samples, which shows that LPS transfer from LptC to LptA required energy (Fig. 4A). In the absence of vanadate, LptC-cross-linked adducts with LPS were not decreased even when LPS transferred to LptA, which suggests that the binding sites for LPS in the periplasmic bridge are always filled with LPS during its transport. Thus, at least two energy-dependent steps are involved in LPS transport: one during extraction from the membrane to the periplasmic domain of the membrane-bound LptC, and another during transfer of LPS



from LptC to LptA. These steps may occur synchronously. We propose a model for LPS transport in which ATP hydrolysis in the cytoplasm provides energy to push LPS molecules in discrete steps across the bridge (Fig. 4B).

Fig. 2. LPS cross-linking in LptA and LptC depends on the inner-membrane components of the Lpt machine. **(A)** LPS accumulates near residue Thr⁴⁷ of LptC in LptBFGC complexes. LptC mutants that cross-link to LPS were overexpressed with or without co-overexpression of LptBFG. Cross-linking was detected similarly to Fig. 1B with antibodies to LPS and LptC. Cross-linking at position 47 substantially increases when LptBFG is co-overexpressed. **(B)** LPS accumulates at several positions of LptA in a LptBFGC-dependent manner. In a method analogous to that in (A), LptA mutants that cross-link to LPS were overexpressed with or without co-overexpression of LptBFGC. Cross-linking at all five positions increases when LptBFGC is co-overexpressed.

The mechanism of LPS transport is somewhat similar to that of efflux pumps, which also form transenvelope bridges and use energy to drive a small molecule through a continuous channel to the exterior environment (26–28). The model

for efflux pumps suggests that only one energy-dependent step drives one toxic molecule out of the cell. This requirement reflects the fact the noxious molecules are released from the internal three-dimensional reservoir into the channel of the pump, which is continuous with the infinite exterior environment; diffusion can efficiently carry the molecule away. Movement of LPS from the inner membrane to the outer membrane across the periplasm proceeds through a series of energy-dependent steps as sequential molecules are pushed in a continuous stream through the bridge. The problem of LPS transport is different from drug efflux in that LPS must move from a two-dimensional reservoir at the inner membrane to another two-dimensional reservoir at the cell surface in opposition to the concentration gradient. Multiple rounds of ATP hydrolysis are required to increase the efficiency of unidirectional transport. If transport across the periplasmic bridge requires that multiple binding sites on Lpt proteins be filled with LPS during transport, it may be possible to design inhibitors that destroy the pumping mechanism by introducing air in the line.

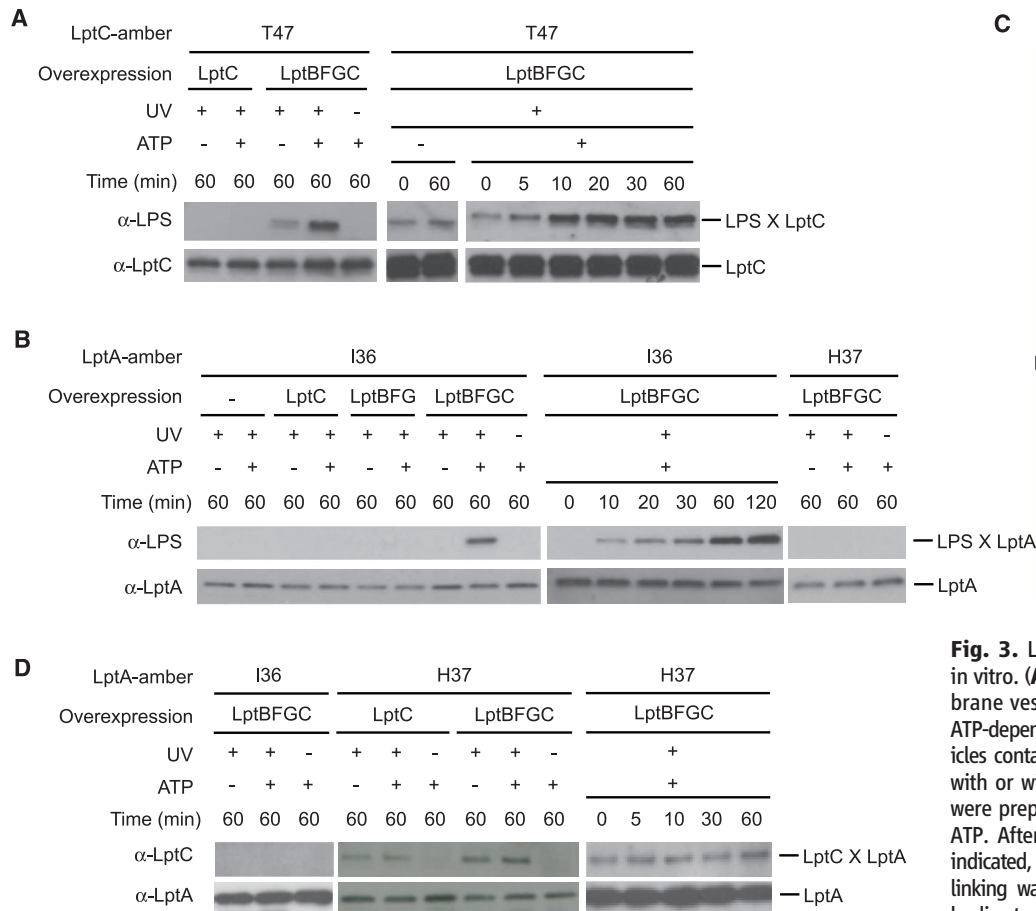


Fig. 3. LPS transport can be reconstituted in vitro. **(A)** LPS accumulates in LptC in membrane vesicles over time in an LptBFG- and ATP-dependent manner. RSO membrane vesicles containing overexpressed LptC(T47pBPA) with or without co-overexpression of LptBFG were prepared in the presence or absence of ATP. After incubation at 30°C for the times indicated, the vesicles were UV-irradiated. Cross-linking was detected as in Fig. 1B with antibodies to LPS and LptC. **(B)** LPS is released to

LptA in an LptBFGC-, time-, and ATP-dependent manner. RSO membrane vesicles were prepared from wild-type cells or cells overexpressing LptC, LptBFG, or LptBFGC with or without added ATP. Purified LptA(I36pBPA) or LptA(H37pBPA) was added to these vesicles, incubated at 30°C for the times indicated, and then UV-irradiated. **(C)** Model of the stacked crystal structures of LptC (green) and LptA (purple). Residues Ile³⁶ and His³⁷ in LptA, which interact with LPS and LptC, respectively, are depicted as stick structures. **(D)** Periplasmic bridge components properly assemble in vitro. Purified LptA(I36pBPA) or LptA(H37pBPA) was added to LptC- or LptBFGC-enriched RSO membrane vesicles. Samples were incubated at 30°C for the times indicated, and then UV-irradiated. LptA(H37pBPA) cross-linked to LptC in an ATP-, time-, and LptBFG-independent manner.

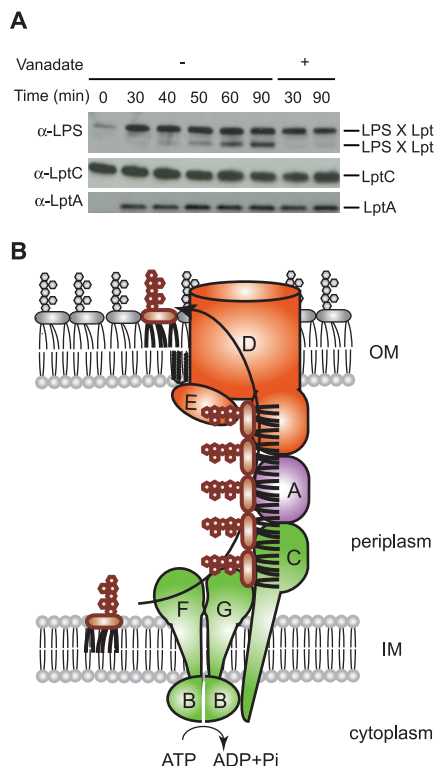


Fig. 4. LPS is pushed in a continuous stream through the Lpt bridge. **(A)** Inhibition of ATP hydrolysis inhibits transfer of LPS from LptC to LptA. RSO membrane vesicles overexpressing LptBFG-LptC (T47pBPA) were incubated at 30°C for 30 min in the presence of ATP to accumulate LPS in LptC. Purified LptA(I36pBPA) was then added with or without vanadate, and the samples were incubated for an additional 60 min. Cross-linking was detected as in Fig. 1B with antibodies to LPS, LptC, and LptA. **(B)** Model of LPS biogenesis. LptBFG extracts LPS from the inner membrane and transports it to LptC using ATP hydrolysis energy. ATP hydrolysis is used again to push LPS from LptC to LptA.

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Supplementary Materials

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Direct Observation of Stalled Fork Restart via Fork Regression in the T4 Replication System

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The restart of a stalled replication fork is a major challenge for DNA replication. Depending on the nature of the damage, different repair processes might be triggered; one is template switching, which is a bypass of a leading-strand lesion via fork regression. Using magnetic tweezers to study the T4 bacteriophage enzymes, we have reproduced in vitro the complete process of template switching. We show that the UvsW DNA helicase in cooperation with the T4 holoenzyme can overcome leading-strand lesion damage by a pseudostochastic process, periodically forming and migrating a four-way Holliday junction. The initiation of the repair process requires partial replisome disassembly via the departure of the replicative helicase. The results support the role of fork regression pathways in DNA repair.

DNA damage causes a replication fork to stall or collapse and is responsible for illegitimate recombination and cellular dysfunction (1, 2). Although damage in the lagging strand is less likely to block fork progression, damage in the leading strand is a particular

challenge because of the continuous nature of the leading-strand synthesis. The fork regression pathway is one means to overcome leading-strand lesions, as generally described in terms of four steps (fig. S1): (i) replisome disassembly; (ii) stalled fork regression to form a Holliday junction

(HJ) structure (also called a chicken-foot structure); (iii) either lesion excision repair or template switching (3, 4), which is polymerase extension of the 3' end of the leading strand, now annealed to an intact template, downstream of the lesion (referred here as lesion bypass); and (iv) restoration of the fork and reloading of the replisome (2). However, the details of such a pathway and the biological role of regressed forks in vivo are poorly understood (2, 5).

The bacteriophage T4 lacks translesion polymerases and, unlike *Escherichia coli*, cannot reinitiate synthesis by repriming the leading strand (6, 7). Hence, the T4 phage may exclusively use

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fork regression to bypass leading-strand lesions via an active helicase-driven pathway involving the UvsW protein (5). UvsW is a functional analog of *E. coli* RecG helicase and has properties similar to those of the RecQ helicase family, which includes human enzymes whose defects contribute to various disease states (8–10). With its dual unwinding and annealing activities (11), UvsW is capable of regressing forks in vitro (5, 6). In the T4 system, when the replisome encounters a leading-strand lesion, replication in the lagging strand continues at least one Okazaki fragment beyond the lesion site (6). This uncoupled synthesis results in a DNA structure that can be effectively regressed by UvsW helicase to generate the HJ structure required for template switching (fig. S1). However, the reconstruction of the full template-switching pathway is missing.

We investigated lesion bypass via template switching, using magnetic tweezers to manipulate a DNA hairpin and follow the activity of the UvsW helicase alone and in collaboration with different components of the replisome. Experiments were carried out by tethering the DNA hairpin between a glass surface and a magnetic bead (Fig. 1A). A pair of magnets was used to apply tension to the ends of the hairpin, while the extension of the DNA molecule $Z_e(t)$ was obtained from tracking the position of the bead (12). Three different hairpins provided either adapted sequences or modified bases (fig. S2): 7-Kbp long (Lh), 1.2-Kbp medium (Mh), and 100-bp short (Sh) hairpins. By applying a force of ~ 15 pN, we trapped Mh molecules in an intermediate configuration in which the initial stem was denatured but the GC-rich region before the apex remained intact (fig. S2D). This partially denatured hairpin was an ideal substrate to simultaneously test UvsW annealing and unwinding activities (Fig. 1B). The changes in $Z_e(t)$ were converted to the number of unwound or annealed base pairs, using the single-stranded DNA (ssDNA) elasticity (fig. S3).

In the presence of UvsW and adenosine triphosphate (ATP), we observed events corresponding to the zipping of the hairpin, followed by the spontaneous recovery of the initial molecular extension (Fig. 1C). After the annealing burst, UvsW remained bound, maintaining the formed hairpin for a fraction of a second before dissociating. The rate of annealing and the enzyme's processivity measured at 15 pN were very large: ~ 1300 bp/s and ~ 9 Kbp, respectively (Fig. 1D and fig. S4). UvsW annealing activity was also tested with an ensemble fluorescence resonance energy transfer-based assay (fig. S5). We found that the UvsW annealing activity is an active process that requires ATP hydrolysis, with a Michaelis constant and catalytic constant of $57 \mu\text{M}$ and 1280 bp/s, respectively (fig. S6). Unwinding by UvsW was observed only when annealing was prevented (closed hairpin at low forces, fig. S7) or in resolving branched structures requiring combined annealing and unwinding activities (fig. S8).

These results showed that UvsW possessed both unwinding and annealing activities, but favored annealing.

Ensemble kinetic studies using a synthetic branched HJ (13) confirmed that UvsW resolved branched structures efficiently (fig. S9). To investigate in real time the generation and migration of an HJ by UvsW with magnetic tweezers, we constructed a DNA substrate mimicking a stalled fork (Fig. 2A) in situ by using either a Mh or Lh (figs. S2 and S10A). The fork regression was then initiated by introducing UvsW and ATP. HJ migration was followed by monitoring changes in $Z_e(t)$ (Fig. 2B). The transient decreases and increases in $Z_e(t)$ corresponded to the migration of the formed HJ in the downward and upward directions. By using double-stranded DNA (dsDNA) elasticity (fig. S3), we converted $Z_e(t)$ into migrated base pairs and measured a rate of HJ migration close to the annealing rate, 1000 to 1300 bp/s, with a small force-dependent asymmetry between the branch migration rates against or favored by the applied force (Fig. 2C and fig. S10). Frequent instantaneous random switches in the migration direction, mediated by a single enzyme complex (figs. S11 and S12), were observed with a characteristic time of ~ 2 s (Fig. 2D). The ability of

UvsW to switch migration direction is essential during the remodeling of stalled forks, because it provides the means to revert the HJ back to a normal fork.

The template-switching pathway (fig. S1) requires the coordinated action of several proteins. We investigated whether the minimal system consisting of UvsW and the T4 holoenzyme [composed of gp43 polymerase, a gp45 sliding clamp, and a gp44/62 clamp loader (14)] was sufficient to reproduce such a pathway in vitro. Ensemble experiments on a plasmid substrate had shown that a lesion in the leading strand regressed a fork by the minimal protein system (6). We prepared a stalled fork substrate presenting a lesion on the leading strand (Fig. 3A), using a 100-bp hairpin with a built-in primer (fig. S13), which contained three locked nucleic acid (LNA) nucleotides in the template leading strand (40 bp before the apex), presenting an efficient roadblock for the holoenzyme (15) that arrested 98% of the molecules (Fig. 3C).

The addition of UvsW, T4 holoenzyme, ATP, and nucleotides first produced a transient decrease associated with fork regression, and next an increase associated with the recovery of the replication fork (Fig. 3B). When the HJ structure

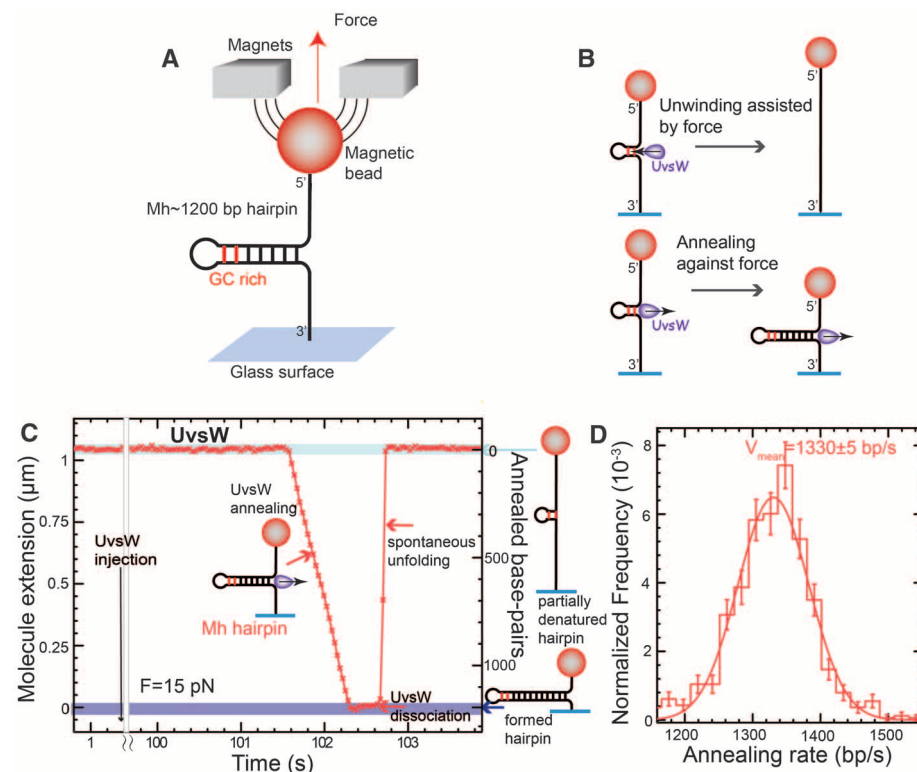


Fig. 1. Single-molecule characterization of UvsW annealing activity. (A) Schematic representation of the experimental setup. A DNA hairpin substrate was tethered between the glass surface and a magnetic bead held in a magnetic trap (MT). The GC-rich region in the Mh is shown in red. (B) Schematics of the assay allowing for unwinding and annealing detection. (C) Experimental trace showing $Z_e(t)$ obtained in the MT assays with the partially denatured hairpin at a force (F) of 15 pN with ATP and UvsW. Annealing bursts were detected as decreases in Z_e . (D) Distribution of annealing rates dZ_e/dt ($n = 766$ events) with a Gaussian fit. Error bars are inversely proportional to the square root of the number of points for each bin.

is formed, the holoenzyme has the ability to extend the leading strand using the exogenous oligonucleotide as the template. We did not directly detect this initial elongation of the primer, because it was not associated with a change in $Z_e(t)$, but its further extension by the holoenzyme after fork progression by UvsW had restored the fork, with the LNA lesion bypassed (Fig. 3B). Template switching and final hairpin synthesis were further confirmed by checking the disappearance of spontaneous fluctuations of the hairpin caused by its conversion to a full DNA

duplex (fig. S14). The frequency of full replicated substrates was increased by more than a factor of 30 in the presence of UvsW (Fig. 3C), confirming the need for fork regression and thus demonstrating the template-switching process in vitro. The fact that the time required for lesion bypass (~ 0.3 s) was much smaller than the typical holoenzyme loading time (~ 20 s) (fig. S15) suggests that the holoenzyme probably remained bound to execute both HJ primer extension and final strand displacement synthesis. The random switching of UvsW between fork regres-

sion and progression appears to be a simple means to coordinate its action with the holoenzyme, without the need for a strict synchronization mechanism.

How does UvsW coordinate its action with the different replisome components so as not to interfere with the normal replication process? First, we tested the effect of T4 gp32 ssDNA binding protein (16) on UvsW activity on a partially denatured hairpin. Normal annealing activity was detected in those assays (fig. S16), demonstrating the ability of UvsW to catalyze protein displacement. Next, we performed competition experiments between UvsW and the holoenzyme and/or replicative helicase. We found that UvsW annealed a partially replicated hairpin even when the T4 holoenzyme was replicating the leading strand, supporting the hypothesis that UvsW annealing promotes the shift of the leading-strand holoenzyme from the fork to the formed HJ (fig. S17A). In contrast, no UvsW activity was detected when the replicative helicase (17) was unwinding the dsDNA (moving along the lagging strand), isolated from or coupled to the T4 holoenzyme during leading-strand synthesis (fig. S17, B and C), demonstrating that gp41 helicase dissociation was required for initiation of the UvsW-catalyzed reversal. Thus, UvsW activity was inhibited during coupled replication (fig. S17D). Blockage of the leading-strand holoenzyme at the lesion site leads to the helicase uncoupling from the holoenzyme and to helicase dissociation [fig. S18 and (6)], then allowing for UvsW loading.

Following the activities of UvsW together with those of the replisome, we have reconstructed the steps for the complete template-switching pathway in vitro. The overall results provide the basis for a stepwise model of a lesion bypass mechanism in T4 (fig. S19): (i) DNA lesion-induced partial uncoupling of the replisome, which allows UvsW loading after the departure of the helicase; (ii) fork regression by UvsW, shifting the leading-strand holoenzyme to the formed HJ; (iii) leading-strand primer extension by the holoenzyme while UvsW is migrating the HJ; (iv) UvsW randomly switching direction; and (v) fork progression by UvsW to recover the replication fork where the lesion is now bypassed. Several helicases, such as *E. coli* RecG or human BLM and WRN

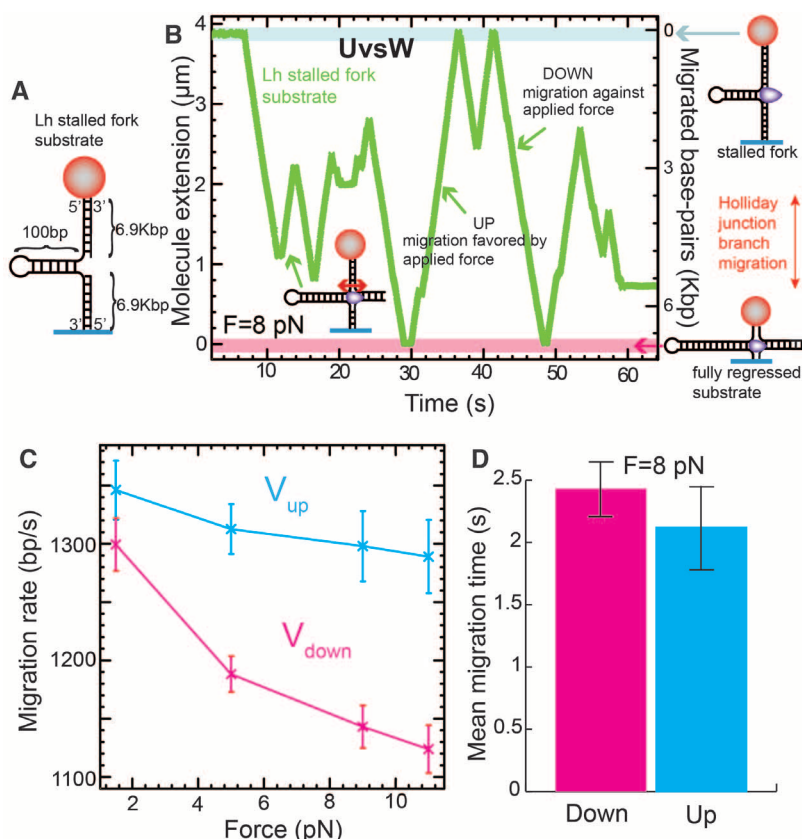
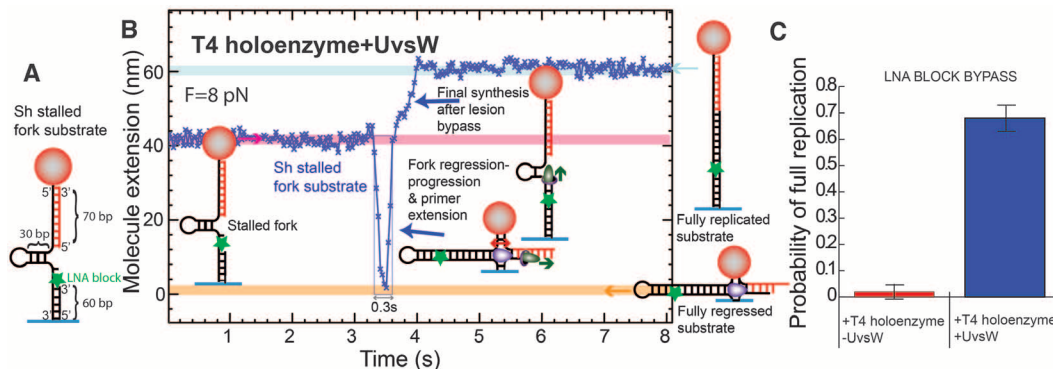


Fig. 2. Single-molecule characterization of UvsW HJ migration activity. (A) Schematics of the Lh stalled fork substrate. (B) Experimental HJ migration trace showing $Z_e(t)$ with the Lh stalled fork substrate, ATP, and UvsW at $F = 8$ pN. (C) Mean HJ migration rate measured in the downward (against the applied force) and upward (favored by the applied force) migration directions as a function of the applied force ($n = 54$ to 248 events). Error bars are SEM. (D) Mean migration time in the downward and upward directions ($n = 69$ events). Error bars are SEM.

Fig. 3. Reconstruction of the template-switching pathway. (A) Sh stalled fork substrate with a LNA block at the leading strand. (B) Experimental trace at $F = 8$ pN showing $Z_e(t)$ with ATP, dNTPs, UvsW, and the T4 holoenzyme, starting with the stalled fork and ending with the fully replicated substrate. (C) Frequency of events showing the full synthesis of the hairpin in the presence ($n = 58$ events) and absence ($n = 95$ events) of UvsW. Error bars are SEM.



helicases, have the ability to remodel stalled forks (18, 19) and can potentially play the role of UvsW in fork regression pathways in *E. coli* and human cells, respectively.

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Supplementary Materials

www.sciencemag.org/cgi/content/full/338/6111/1217/DC1
Materials and Methods
Figs. S1 to S19
Table S1
References (20–28)

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Progenitor and Terminal Subsets of CD8⁺ T Cells Cooperate to Contain Chronic Viral Infection

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Chronic infections strain the regenerative capacity of antiviral T lymphocyte populations, leading to failure in long-term immunity. The cellular and molecular events controlling this regenerative capacity, however, are unknown. We found that two distinct states of virus-specific CD8⁺ T cells exist in chronically infected mice and humans. Differential expression of the T-box transcription factors T-bet and Eomesodermin (Eomes) facilitated the cooperative maintenance of the pool of antiviral CD8⁺ T cells during chronic viral infection. T-bet^{hi} cells displayed low intrinsic turnover but proliferated in response to persisting antigen, giving rise to Eomes^{hi} terminal progeny. Genetic elimination of either subset resulted in failure to control chronic infection, which suggests that an imbalance in differentiation and renewal could underlie the collapse of immunity in humans with chronic infections.

Lifelong immunity to acutely resolved infections requires maintenance of memory lymphocytes that, upon reinfection, regenerate a large cohort of short-lived, terminal progeny while concurrently replenishing the long-lived memory pool. During low-level latent/reactivating infections, such as cytomegalovirus (CMV), this periodic replenishment of short-lived and long-lived populations is balanced to achieve lifelong immunity. Chronic viral in-

fections with prolonged and elevated antigen load, however, may strain the renewal capacity of long-lived lymphocytes by persistent elicitation of short-lived cells. For example, increased CD8⁺ T cell turnover is observed during HIV infection (1–3). This stems from an elevated production of short-lived cells and a concurrent reduction in the long-lived pool (4) and is thought to underlie the eventual collapse of adaptive immunity during HIV infection. It is unclear, however, which molecular pathways govern the renewal and differentiation of virus-specific CD8⁺ T cells during chronic infections, or whether these antiviral responses are sustained by a progenitor-progeny relationship.

Two T-box transcription factors, T-bet and Eomesodermin (Eomes), regulate both functional and dysfunctional CD8⁺ T cell responses (5–10). During chronic viral infections, T-bet is reduced in virus-specific CD8⁺ T cells, and this reduction correlates with T cell dysfunction (9–11). In contrast, microarray analysis of CD8⁺ T cells suggested that Eomes mRNA is elevated during

chronic infection (12). Using acute or chronic infection with lymphocytic choriomeningitis virus (LCMV), we found that Eomes expression was up-regulated in exhausted CD8⁺ T cells during chronic infection (Fig. 1, A to C, and fig. S1A).

In contrast to acute infection, T-bet and Eomes were reciprocally expressed in exhausted CD8⁺ T cells by day 15 to day 30 of chronic infection (Fig. 1D). In addition, Eomes-expressing CD8⁺ T cells had higher expression of Blimp-1 and several inhibitory receptors (Fig. 1, E and F, and fig. S1B), consistent with more severe exhaustion (13–17). Furthermore, Eomes^{hi} virus-specific CD8⁺ T cells had less co-production of interferon- γ (IFN- γ) and tumor necrosis factor- α (TNF- α) (16, 18) (fig. S1C). However, high expression of Eomes or the inhibitory receptor PD-1 correlated with increased granzyme B and cytotoxicity, despite lower degranulation (fig. S1, D to H). Thus, Eomes expression in virus-specific CD8⁺ T cells during chronic infection was associated with markers of severe exhaustion and reduced co-production of antiviral cytokines, despite better cytotoxicity than T-bet^{hi} cells.

Although wild-type CD8⁺ T cells had bimodal expression of T-bet and Eomes, genetic deletion of T-bet resulted in increased Eomes and PD-1 expression (Fig. 1G) (9). In contrast, Eomes-deficient CD8⁺ T cells had reduced PD-1 and Blimp-1, as well as increased T-bet and cytokine co-production (Fig. 1, G to I, and fig. S2). Thus, T-bet and Eomes support separate subpopulations of virus-specific CD8⁺ T cells during chronic infection.

To determine the relative contribution of these T-bet^{hi} and Eomes^{hi} CD8⁺ T cell subsets to the magnitude of the response during chronic infection, we quantified these populations in multiple anatomical locations. Overall, the Eomes^{hi} population outnumbered the T-bet^{hi} population by a factor of ~20 (fig. S3A), and these subsets had distinct tissue distribution (fig. S3, B and C). As observed in intact mice, sorted and adoptively transferred PD-1^{int} (T-bet^{hi}) cells preferentially accumulated in the spleen, whereas PD-1^{hi}

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(Eomes^{hi}) cells distributed systemically (fig. S3, D and E). These data indicate a large numerical accumulation of Eomes^{hi} exhausted CD8⁺ T cells

throughout the body and suggest potential distinct contributions of Eomes^{hi} and T-bet^{hi} subsets to the antiviral response.

Previous studies suggest that proliferating and nonproliferating subpopulations of exhausted CD8⁺ T cells exist during chronic infections (4, 19),

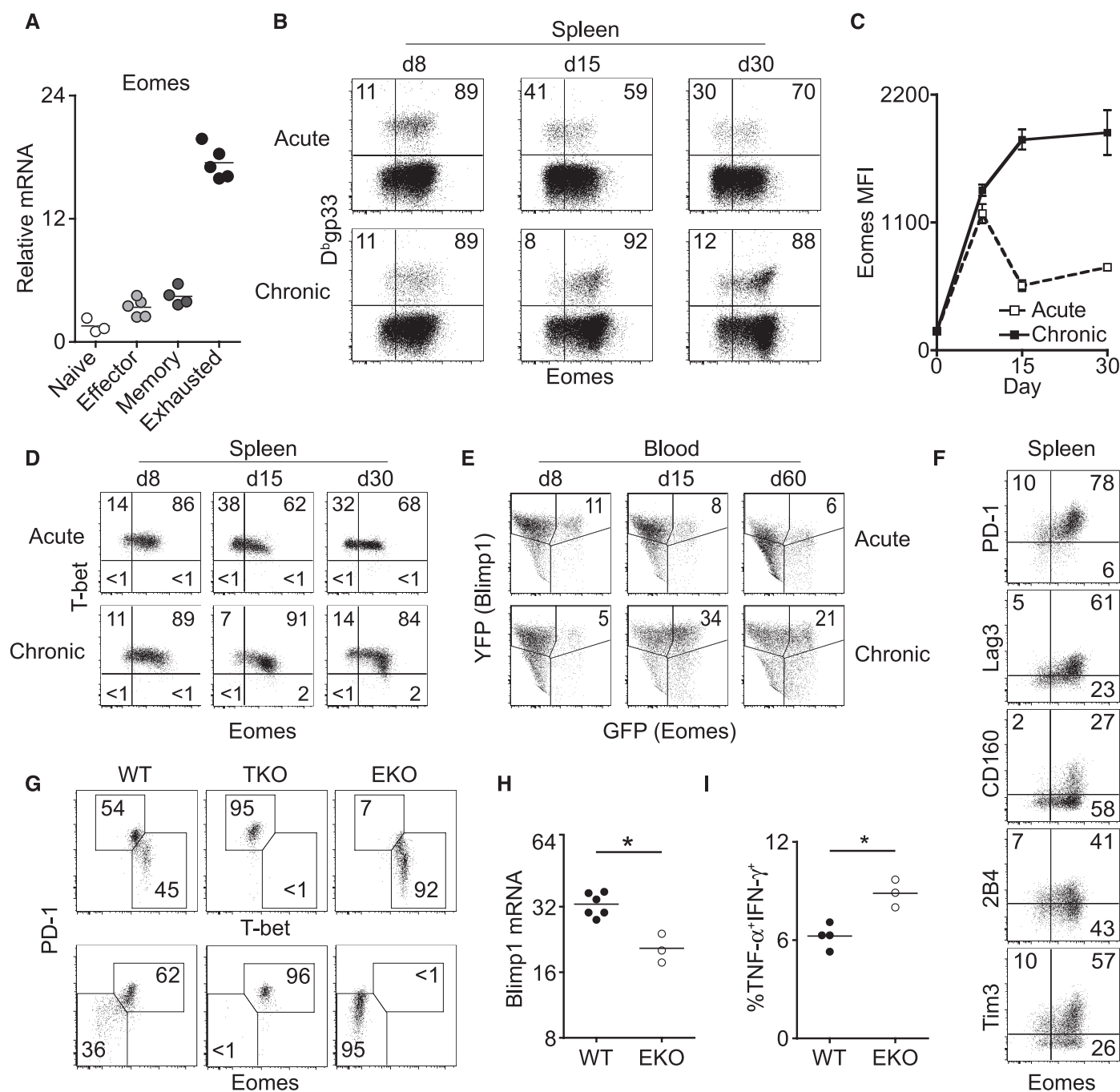


Fig. 1. Eomes expression is up-regulated during chronic viral infection and supports enhanced CD8⁺ T cell exhaustion. (A) Sorted naïve or D^bgp276-specific CD8⁺ T cells from effector [acute; Armstrong day 8 (d8)], memory (acute; Armstrong d30), or exhausted (chronic; clone 13, d30) time points were analyzed for Eomes mRNA expression by real-time quantitative polymerase chain reaction. (B) Flow cytometric analysis of Eomes protein expression in D^bgp33-specific CD8⁺ T cells at indicated numbers of days post-infection (p.i.). (C) Mean fluorescence intensity (MFI) of Eomes expression from cells in (B). Graph displays means $\pm$ SEM. (D) Flow cytometric analysis of T-bet versus Eomes expression in D^bgp33-specific CD8⁺ T cells at indicated days p.i. Gates are based on T-bet⁻negative and Eomes⁻negative cells. (E) Longitudinal frequency of GFP⁺YFP⁺ D^bgp33-specific CD8⁺ T cells in Blimp1-YFP/Eomes^{d/p/+}

dual reporter mice as analyzed by flow cytometry (GFP and YFP, green and yellow fluorescent protein, respectively). (F) Flow cytometric analysis of inhibitory receptor versus Eomes expression in D^bgp33-specific CD8⁺ T cells at d30 p.i. (G) Flow cytometric analysis of T-bet, Eomes, and PD-1 expression of D^bgp33-specific CD8⁺ T cells from wild-type (WT), T-bet KO (TKO), and Eomes cKO (EKO) mice at d22 p.i. (H) Blimp1 relative mRNA in D^bgp276-specific CD8⁺ T cells from WT and EKO mice at d15 p.i. (* P < 0.05; Mann-Whitney). (I) IFN-γ and TNF-α expression in CD8⁺ T cells from indicated mice after peptide pool stimulation as in fig. S1 (** P < 0.01; unpaired t test). In (B) to (G), numbers denote frequency of gated population; in (F) to (I), cells are from clone 13 infection. All data are representative of two to five independent experiments with at least three mice per experimental group.

but the regulation of this process is poorly understood. We hypothesized that T-bet^{hi} and Eomes^{hi} CD8⁺ T cells might have different in vivo proliferative dynamics. Whereas T-bet^{hi} CD8⁺ T cells had low expression of the cell cycle-regulated protein Ki67 and low incorporation of 5-bromo-2'-deoxyuridine (BrdU), Eomes^{hi} CD8⁺ T cells expressed Ki67 and had high BrdU incorporation in multiple tissues (Fig. 2A and fig. S4, A to C). Using carboxyfluorescein succinimidyl ester (CFSE) (fig. S4D), we observed robust proliferation in CD8⁺ T cells that were Eomes^{hi} at the time of analysis, whereas less CFSE dilution was

observed for the T-bet^{hi} population (Fig. 2B). The modest proliferation of T-bet^{hi} cells was unlikely to have been due to limited access to antigen, because essentially all virus-specific memory CD8⁺ T cells divided extensively in this setting (fig. S4, E and F) (19). In addition, T-bet and Eomes governed these distinct proliferative behaviors, because deletion of T-bet increased Ki67 expression and BrdU incorporation, whereas loss of Eomes diminished proliferation (Fig. 2C and fig. S4G). Thus, T-bet and Eomes regulate distinct CD8⁺ T cell pools with differential division during chronic infection.

To trace lineage relationships, we used PD-1 expression as a surrogate marker (fig. S1F) to separate T-bet^{hi} and Eomes^{hi} subsets, followed by CFSE labeling to monitor proliferation and differentiation. After adoptive transfer to infection-matched mice (fig. S5A), PD-1^{hi} (Eomes^{hi}) CD8⁺ T cells divided modestly and retained high expression of PD-1 (Fig. 2D and fig. S5B). In contrast, purified PD-1^{int} (T-bet^{hi}) CD8⁺ T cells demonstrated enhanced in vivo proliferation, and this extensive division was associated with conversion to PD-1^{hi} (Fig. 2D and fig. S5B). Similar results were obtained using an *Eomes*^{gfp/+} reporter

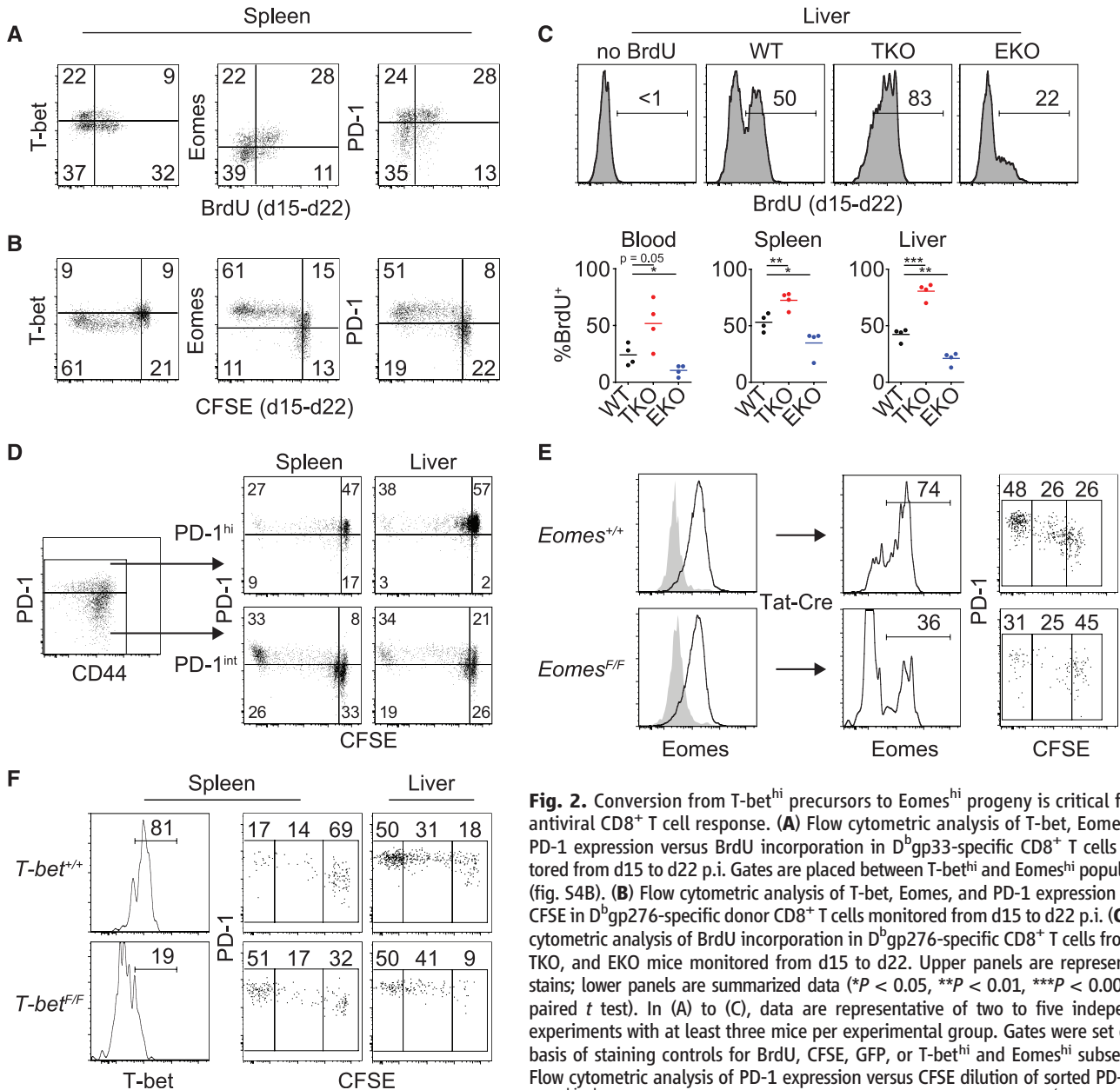


Fig. 2. Conversion from T-bet^{hi} precursors to Eomes^{hi} progeny is critical for the antiviral CD8⁺ T cell response. (A) Flow cytometric analysis of T-bet, Eomes, and PD-1 expression versus BrdU incorporation in D^bgp33-specific CD8⁺ T cells monitored from d15 to d22 p.i. Gates are placed between T-bet^{hi} and Eomes^{hi} populations (fig. S4B). (B) Flow cytometric analysis of T-bet, Eomes, and PD-1 expression versus CFSE in D^bgp276-specific donor CD8⁺ T cells monitored from d15 to d22 p.i. (C) Flow cytometric analysis of BrdU incorporation in D^bgp276-specific CD8⁺ T cells from WT, TKO, and EKO mice monitored from d15 to d22. Upper panels are representative stains; lower panels are summarized data (**P* < 0.05, ***P* < 0.01, ****P* < 0.001; unpaired *t* test). In (A) to (C), data are representative of two to five independent experiments with at least three mice per experimental group. Gates were set on the basis of staining controls for BrdU, CFSE, GFP, or T-bet^{hi} and Eomes^{hi} subsets. (D) Flow cytometric analysis of PD-1 expression versus CFSE dilution of sorted PD-1^{int} or PD-1^{hi} D^bgp276-specific CD8⁺ T cells 7 days after transfer. (E) *Eomes*^{+/+} and *Eomes*^{F/F}

CD8⁺ T cells were isolated at d15 p.i. and treated in vitro with Tat-Cre. Two weeks after transfer, D^bgp276-specific CD8⁺ T cells were assessed for Eomes and PD-1 expression and CFSE dilution by flow cytometry. (F) *T-bet*^{+/+} and *T-bet*^{F/F} CD8⁺ T cells were isolated at d15 p.i. and treated in vitro with Tat-Cre. Two weeks after transfer, D^bgp276-specific CD8⁺ T cells were assessed for T-bet and PD-1 expression and CFSE dilution by flow cytometry. Data in (D) to (F) are representative of two to four independent experiments.

mouse (20) (fig. S5, C and D). Thus, virus-specific CD8⁺ T cells converted from T-bet^{hi} to Eomes^{hi} in a process coupled to extensive cell division. Once generated by conversion from T-bet^{hi} cells, however, the PD-1^{hi} Eomes^{hi} T-bet^{lo} subset had reduced capacity to undergo additional vigorous proliferation in vivo.

To test whether Eomes was essential during the T-bet^{hi} to Eomes^{hi} transition, we treated virus-specific CD8⁺ T cells from chronically infected *Eomes*^{+/+} and *Eomes*^{F/F} mice in vitro with Tat-Cre and adoptively transferred them into infection-matched mice (fig. S5E). The temporal loss of Eomes reduced the extensively divided CD8⁺ T cell population (Fig. 2E), which suggests that Eomes is critical for initiating or sustaining this proliferative and conversion event. In contrast, temporal deletion of T-bet (fig. S5F) accelerated decay of the progenitor population (Fig. 2F), suggesting a critical role for T-bet in maintaining this precursor pool. Thus, temporal deletion indicates an ongoing requirement for these transcription factors in regulating the differential proliferative behavior of subsets of exhausted CD8⁺ T cells.

The absence of T-bet impedes the control of chronic viral infection, possibly due to a shift toward more severe exhaustion (9). In light of the reduced features of exhaustion when Eomes was deleted (Fig. 1, G to I), we tested whether eliminating Eomes would improve control of chronic viral infection. In the absence of T-bet or Eomes, however, virus-specific CD8⁺ T cells could neither maintain the antiviral response (Fig. 3, A and B, and figs. S6 and S7) nor limit viral replication (Fig. 3C and fig. S8). Because each subpopulation failed to independently sustain an effective response, it was possible that the T-bet-dependent and Eomes-dependent subsets might contribute distinct functions and

cooperate to achieve viral control. To test this possibility, we examined mixed chimeras containing both Eomes conditional knockout (cKO) and T-bet KO bone marrow. The combination of Eomes cKO (T-bet^{hi})–exhausted and T-bet KO (Eomes^{hi})–exhausted CD8⁺ T cells, however, did not improve viral control over either subset alone (Fig. 3D). These data suggest that the major factor sustaining virus-specific CD8⁺ T cell responses and containing viral replication during chronic infection is not independent functions of T-bet^{hi} and Eomes^{hi} CD8⁺ T cells, but rather the lineage relationship between these subpopulations.

We next tested whether persisting antigen was necessary for converting T-bet^{hi} precursors into the Eomes^{hi} progeny. Relative to cells adoptively transferred into a wild-type infection, transfer into mice chronically infected with a variant of LCMV containing a mutation in the gp33-41 epitope (V35A) (19) resulted in H-2D^b-restricted gp33-specific CD8⁺ T cells with elevated T-bet but reduced Eomes and PD-1 expression (Fig. 4A). The preferential recovery of T-bet^{hi} cells in the absence of antigen resulted from poor persistence of preformed Eomes^{hi} cells and reduced repopulation of Eomes^{hi} cells from the T-bet^{hi} pool (Fig. 4B and fig. S9). In addition, Eomes^{hi} cells retained Eomes expression and did not revert back to Eomes^{lo} cells after the removal of antigen (fig. S9C), which suggested that conversion from T-bet^{hi} to Eomes^{hi} during chronic infection is accompanied by terminal differentiation.

If persisting antigen induces T-bet^{hi} precursors to continually proliferate and give rise to Eomes^{hi} progeny, one might predict that prolonged, uncontrolled viral replication could eventually deplete the T-bet^{hi} precursor population. This possibility was tested by transiently deplet-

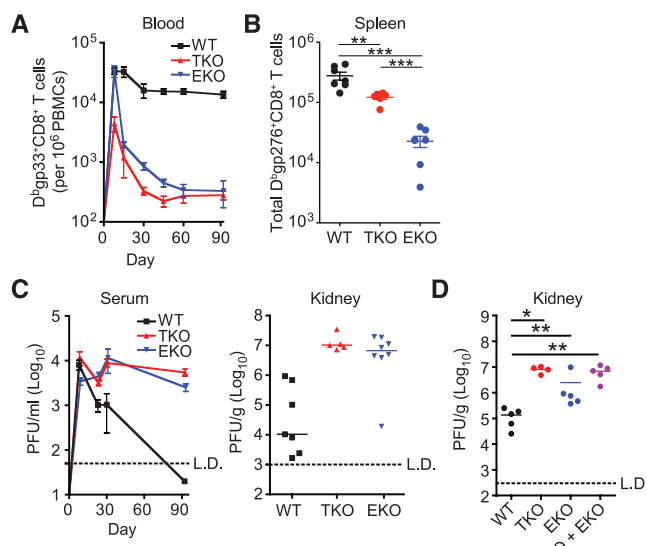
ing CD4⁺ T cells before LCMV clone 13 infection, which led to lifelong high viral load (21). Sustained high viral loads caused an erosion of the in vivo BrdU accumulation in the Eomes^{hi} subset and loss of T-bet^{hi} precursors over time (Fig. 4, C and D, and fig. S10), suggesting a progressive imbalance of T-bet^{hi} to Eomes^{hi} lineage repopulation.

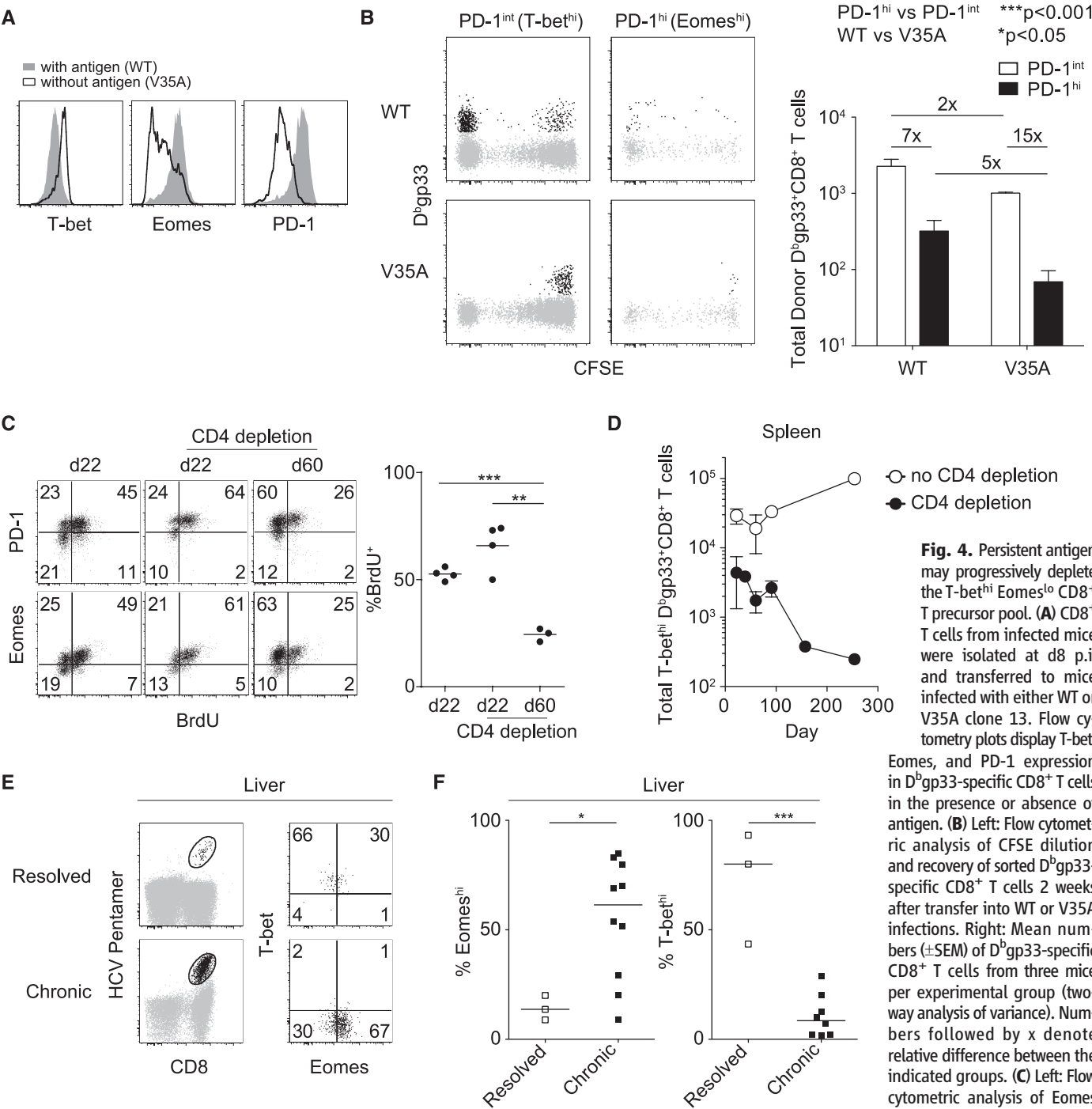
To examine whether years of chronic viral infection erode virus-specific CD8⁺ T cell renewal capacity by continuous depletion of the T-bet^{hi} precursor pool, we examined hepatitis C virus (HCV) infection. Although 20 to 30% of HCV infections spontaneously resolve, most untreated patients experience a high viral burden for many years (22). Patients with uncontrolled viral replication have a gradual loss of systemic CD8⁺ T cell responses with an associated accumulation of highly exhausted T cells in the liver (23–26), consistent with a dysregulated balance between self-renewal and terminal differentiation. We therefore examined whether T-bet^{hi} precursors could be found in patients with resolved versus chronic HCV infection and whether Eomes^{hi} progeny accumulated in patients with active viral replication (Fig. 4E). Although HCV-specific CD8⁺ T cells in the blood trended toward higher Eomes expression in chronically infected subjects, there was equivalent T-bet expression in systemic responses from resolved or chronic infection (fig. S11). In contrast, at the site of viral replication, there was substantial accrual of Eomes^{hi} HCV-specific CD8⁺ T cells and a relative depletion of the T-bet^{hi} subset during chronic infection relative to resolved infection (Fig. 4, E and F). Consistent with the observations in mice, chronic HCV infection is associated with few T-bet^{hi} precursors and concurrent accumulation of Eomes^{hi} terminal progeny.

Memory CD8⁺ T cells generated after acutely resolved infection or successful vaccination are quiescent, but they are capable of slow self-renewal and production of differentiated effector progeny upon reinfection (27, 28). In contrast, exhausted CD8⁺ T cells during chronic infections are continually activated by persisting antigen and undergo prolonged, extensive division (19, 29, 30). It has been unclear how the regenerative capacity of these cells develops and why it ultimately fails in pathological chronic infections. Collectively, our results suggest that in the face of persisting antigen, virus-specific CD8⁺ T cells make use of two homologous T-box transcription factors to maintain long-lasting antiviral immunity. This maintenance parallels progenitor-progeny dynamics of cells in other tissues organized according to proliferative hierarchies. Although unable to fully eradicate the virus, these two cell subsets act together to maintain a durable and partially effective CD8⁺ T cell response during chronic infection.

Despite functional impairment, exhausted CD8⁺ T cells are not inert. Rather, these cells

Fig. 3. Deletion of T-bet or Eomes leads to impaired maintenance of the CD8⁺ T cell response and loss of viral control. (A) Longitudinal frequency of D^bgp33-specific CD8⁺ T cells in the blood of infected mice (PBMCs, peripheral blood mononuclear cells). (B) Total D^bgp276-specific CD8⁺ T cells in the spleens of indicated mice at d30 p.i. (***P* < 0.01, ****P* < 0.001; unpaired *t* test). (C) Viral load in the serum longitudinally and kidney at d90 p.i. (PFU, plaque-forming units). (D) Viral load in kidneys of bone marrow chimeras transplanted with indicated bone marrow at d90 p.i. (**P* < 0.05, ***P* < 0.01; Mann-Whitney). All panels used LCMV clone 13 infection. Data are representative of two to six independent experiments with at least four mice per experimental group.





incorporation in D^bgp276-specific CD8⁺ T cells from WT mice infected with clone 13 with or without CD4⁺ T cell depletion. Right: Summary data for multiple mice (**P < 0.01, ***P < 0.001; unpaired t test). Data in (A) to (C) are representative of two or three independent experiments with at least three mice per experimental group. (D) Total T-bet^{hi} D^bgp33-specific CD8⁺ T cells were enumerated at the indicated time points with or without CD4⁺ T cell depletion. Data are aggregated across three independent experiments. (E) Flow cytometric analysis of T-bet and Eomes expression in intrahepatic HCV-specific CD8⁺ T cells isolated from patients with resolved or chronic infections. (F) Frequency of Eomes^{hi} and T-bet^{hi} HCV-specific CD8⁺ T cells in the liver of patients with resolved or chronic infections (*P < 0.05, ***P < 0.001; unpaired t test). In (E) and (F), a total of 3 and 10 samples were analyzed from resolved and chronic infections, respectively.

continually constrain viral replication and/or drive viral epitope escape (31). The critical balance between progenitors and progeny in exhausted CD8⁺ T cells indicates a vital role for proper regulation of antiviral lymphocyte dynamics during chronic infections. In addition, the ability to define and monitor progenitors and progeny over time might provide an opportunity to predict the collapse of long-term control of chronic infections. Delineating the molecular coordination of this process provides a framework for prophylactic or therapeutic strategies to improve the

durability and regenerative capacity of antiviral T cells during persisting infections.

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Supplementary Materials

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Materials and Methods

Figs. S1 to S11

References (32–40)

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Body Cues, Not Facial Expressions, Discriminate Between Intense Positive and Negative Emotions

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The distinction between positive and negative emotions is fundamental in emotion models. Intriguingly, neurobiological work suggests shared mechanisms across positive and negative emotions. We tested whether similar overlap occurs in real-life facial expressions. During peak intensities of emotion, positive and negative situations were successfully discriminated from isolated bodies but not faces. Nevertheless, viewers perceived illusory positivity or negativity in the nondiagnostic faces when seen with bodies. To reveal the underlying mechanisms, we created compounds of intense negative faces combined with positive bodies, and vice versa. Perceived affect and mimicry of the faces shifted systematically as a function of their contextual body emotion. These findings challenge standard models of emotion expression and highlight the role of the body in expressing and perceiving emotions.

Jennifer checks the numbers in her lottery ticket, when she realizes she hit the 10-million-dollar jackpot. Michael fumbles for his car keys while his 3-year-old son steps into the street and is hit by a passing car. In a split second, Jennifer and Michael experience the most intense emotions of their lives. Intuitively, their emotional expressions should differ vastly, an assumption shared by leading models of emotion. For example, basic emotion models, which posit distinctive categories of emotions such as anger and fear, predict that intense emotions activate maximally distinct facial muscles, which increase

discrimination (1, 2). Similarly, dimensional emotion models, which posit that valence is a primary dimension of emotion perception, predict that intense emotions are located on more extreme positions on the pleasure-displeasure axis and thus their positivity or negativity should be easier to decipher (3).

The question of affective valence discrimination is theoretically important for the structure of emotion models and is central for understanding how social communication takes place in highly intense and potentially dangerous situations. Yet, although it is commonly assumed that facial expressions convey positive and negative affective valence in a highly distinct manner, there is still room for question on both methodological and theoretical grounds. From a methodological standpoint, most studies to date have used posed prototypical facial expressions (1) that have been carefully designed to signal clear and distinct emotions (4–7), and indeed the higher their in-

tensity, the more accurately recognized they become (8–10). However, expressive facial behavior may be different in real-life situations. Studies that have shown successful affective valence differentiation using ecological expressions have not focused on the transient peaks of intense emotions. For example, a study on face expressions in the Judo Olympics sampled reactions across a relatively extended duration (~15 s) after the transient emotional peak, potentially diluting the most intense reactions (11). Other work showing good accuracy in differentiating pain and sensual pleasure did not focus on intense emotional peaks altogether (12). Therefore, it remains unclear how distinct peak intensity expressions of opposite affective valence actually are.

Further, a clear-cut distinction between positive and negative expressions may be theoretically unwarranted. Neurobiological work has shown that the opioid and dopamine systems modulate both pain and pleasure (13), and brain imaging studies consistently show regions activated by both positive and negative emotions, including the insula, striatum, orbitofrontal cortex, nucleus accumbens, and amygdala (14–19). Similarly, research in motivation and emotion has shown that a sharp distinction between positive and negative emotion experience is sometimes hard to draw (20–22). These findings all hint at potentially overlapping and shared mechanisms across positive and negative emotions.

We therefore examined whether facial expressions of opposite affective valence might also overlap during highly intense peaks of emotion. We defined a peak emotion as the apex of a highly intense emotional experience and focused on the immediate peak expressions in response to real-life situations such as undergoing a nipple piercing, receiving an extravagant prize, winning a point in a professional sports match, and so forth. Furthermore, unlike most previous work [e.g., (23)], we took a “full person” approach (24) and

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examined the face-body dynamics during expression. We predicted that during peak intensity moments, facial reactions would overlap and be nondiagnostic for the affective valence of the situation. Consequently, we expected that body context would “step in” and aid in disambiguating the face.

To test this hypothesis, we first used peak expressive reactions to winning and losing points in professional high-stakes tennis matches that typically evoke strong affective reactions (experiment 1). Three groups of 15 participants rated the affective valence and intensity of either the full image (face + body), the body alone, or the face alone (Fig. 1, A and B) (25). Consistent with our prediction, whereas participants failed to rate the affective valence of winners as more positive than the affective valence of losers when seeing the face alone, they succeeded when seeing the body alone or the body and the face together [mixed analysis of variance (ANOVA): $F(2, 42) = 74.05$, $P < 0.0001$] (Fig. 1C). Specifically, affective valence was higher for winners than losers when ratings were based on the face + body ($P < 0.0001$), or the body alone ($P < 0.0001$), but not when ratings were based on the face alone ($P > 0.3$). Notably, although faces were not diagnostic for affective valence, they were diagnostic for intensity [mixed ANOVA: $F(2, 42) = 37.5$, $P < 0.0001$] (Fig. 1D). Thus, intensity ratings were higher for winners than losers when based on the face + body ($P < 0.0001$), or the face ($P < 0.0001$), but not when based on the body alone ($P > 0.1$).

Intriguingly, we found an illusion in the perception of the nondiagnostic faces: 53.3% of the participants who completed the perceptual

face + body affective valence rating task reported relying on face cues (which in fact were nondiagnostic), whereas 46.6% reported relying on body cues (no significant difference). Furthermore, among a separate group of participants who were given a description of the type of images in our task (without seeing the stimuli), 80% chose the face as the part that would be most diagnostic for affective valence discrimination, 20% chose the face + body as equally diagnostic, and none chose the body. We refer to this phenomenon as illusory facial affect: the perceptual attribution of clear positive or negative affect to an inherently ambiguous face while disregarding the objective diagnostic source of the affect in the body.

Illusory facial affect hints at a critical role for the body in shaping the perceived affective valence in intense expressions. We tested this proposition directly by creating face-body compounds with photos of losing faces combined with winning bodies, as well as winning faces combined with losing bodies (supplementary text). Participants rated the facial affective valence in these manipulated images, alongside the original images (experiment 2, Fig. 2A). The critical images were diluted amid a large number of filler images, and participants were unaware of the manipulation. As predicted, the perceived affective valence of the same faces shifted categorically depending on the body with which they appeared [repeated ANOVA: body effect $F(1, 14) = 118$, $P < 0.0001$] (Fig. 2B). Indeed, the effect of the body was slightly stronger for the incongruent face combinations, indicating again that the face itself was nondiagnostic

[repeated ANOVA: interaction effect $F(1, 14) = 10.9$, $P < 0.005$].

Furthermore, the nondiagnosticity of faces generalized to a wider range of intense emotional situations (experiment 3, Fig. 3A). Participants rated faces from three intense positive situations [which included joy (seeing one's house after a lavish makeover), pleasure (experiencing an orgasm), and victory (winning a tennis point)] and faces from three negative situations [which included grief (reacting at a funeral), pain (undergoing a nipple or naval piercing), and defeat (losing a tennis point)]. Isolated faces were nondiagnostic for the affective valence of the situation. Indeed, faces from positive events ($M = -1.4$, $SE = 0.16$) were rated as more negative than faces from negative events ($M = -0.82$, $SE = 0.19$) [repeated ANOVA: $F(1, 14) = 29.6$, $P < 0.0001$]. A comparison of the facial reactions within each pair of opposing emotions also failed to demonstrate the correct affective valence direction: joy ($M = -1.40$, $SE = 0.13$) versus grief ($M = -1.42$, $SE = 0.16$), $P > 0.5$; pleasure ($M = -1.42$, $SE = 0.15$) versus pain ($M = -0.07$, $SE = 0.24$), $P < 0.001$; and victory ($M = -1.40$, $SE = 0.24$) versus defeat ($M = -0.9$, $SE = 0.23$), $P < 0.001$.

We further tested the inherent ambiguity of the faces by combining all face exemplars with positive-valence (a victorious body) or negative-valence (a person undergoing piercing) (Fig. 3B) (25). Although the influence of the bodies was stronger for some faces than for others [repeated ANOVA: interaction effect, $F(5, 70) = 4.9$, $P < 0.001$], the effect of the body was significant [repeated ANOVA: body effect, $F(1, 14) = 96.9$, $P < 0.0001$] and held for every pair of emotions

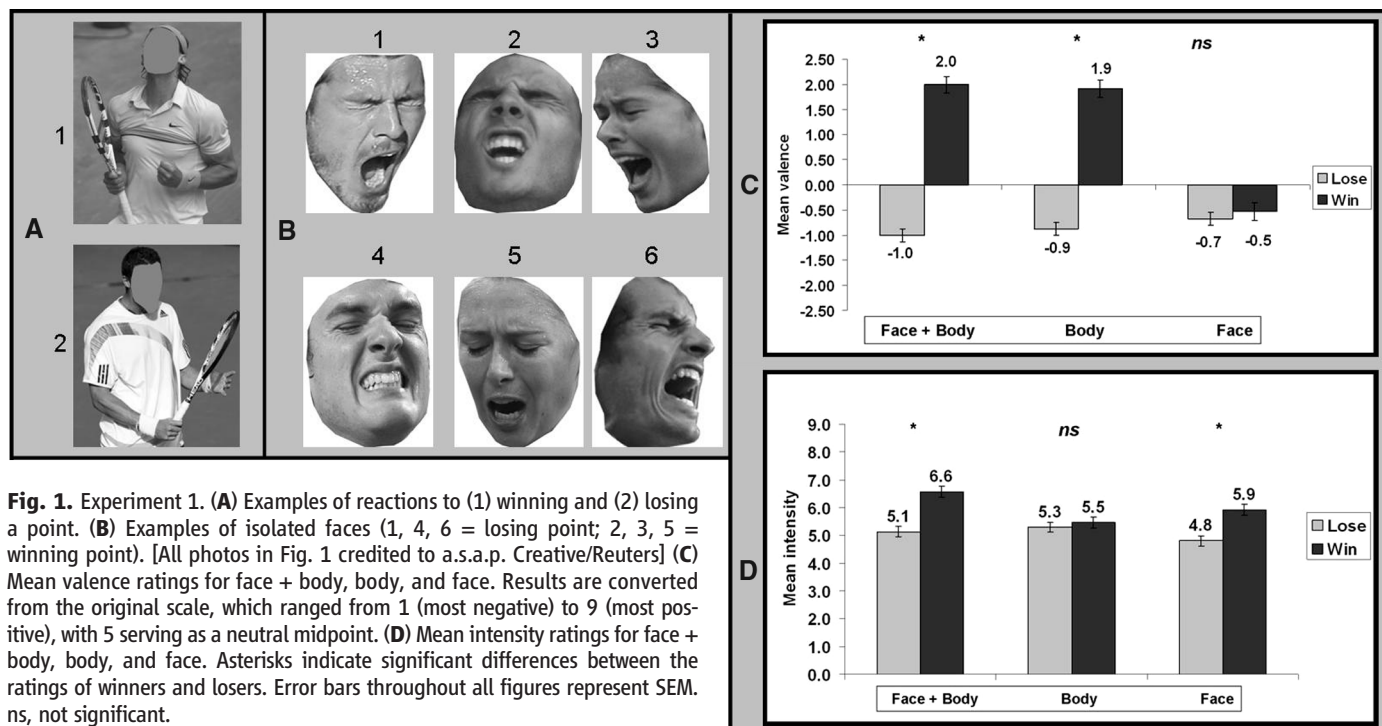


Fig. 1. Experiment 1. (A) Examples of reactions to (1) winning and (2) losing a point. (B) Examples of isolated faces (1, 4, 6 = losing point; 2, 3, 5 = winning point). [All photos in Fig. 1 credited to a.s.a.p. Creative/Reuters] (C) Mean valence ratings for face + body, body, and face. Results are converted from the original scale, which ranged from 1 (most negative) to 9 (most positive), with 5 serving as a neutral midpoint. (D) Mean intensity ratings for face + body, body, and face. Asterisks indicate significant differences between the ratings of winners and losers. Error bars throughout all figures represent SEM. ns, not significant.

[all paired t tests, $P < 0.001$] (Fig. 3C). As the piercing image may have been unusually potent and unambiguous, we further tested the contextual malleability of the joy, grief, pleasure, and pain images, combined with winning and losing bodies. For each of the four face categories, the results fully replicated the categorical face valence shift as a function of the body [repeated ANOVA: body effect, $F(1, 9) = 63.01$, $P < 0.0001$; all paired contrasts, $P < 0.001$]. Thus, intense isolated faces across a broad variety of emotional situations were nondiagnostic for affect-

tive valence, and their perceived positivity or negativity shifted categorically as a function of the body.

Finally, to test if participants' perceptions of the faces actually change depending on the body, we again combined winning faces and losing bodies, and vice versa. However, rather than rating the faces, participants were instructed to pose and simulate in their own face the exact facial movements portrayed by the tennis players (25). If perceptions of the contextualized faces actually change, then we should expect the systematic shift

to extend to the motor simulation of the faces (26, 27).

This study (experiment 4) included two phases. In the "posing phase," participants viewed contextualized faces. The stimuli included (i) winning faces on winning bodies (the original images), (ii) winning faces combined with losing bodies, (iii) losing faces on losing bodies (the original images), and (iv) losing faces combined with winning bodies. As in experiment 2, these critical images appeared amid a large number of filler trials (which were not analyzed) and

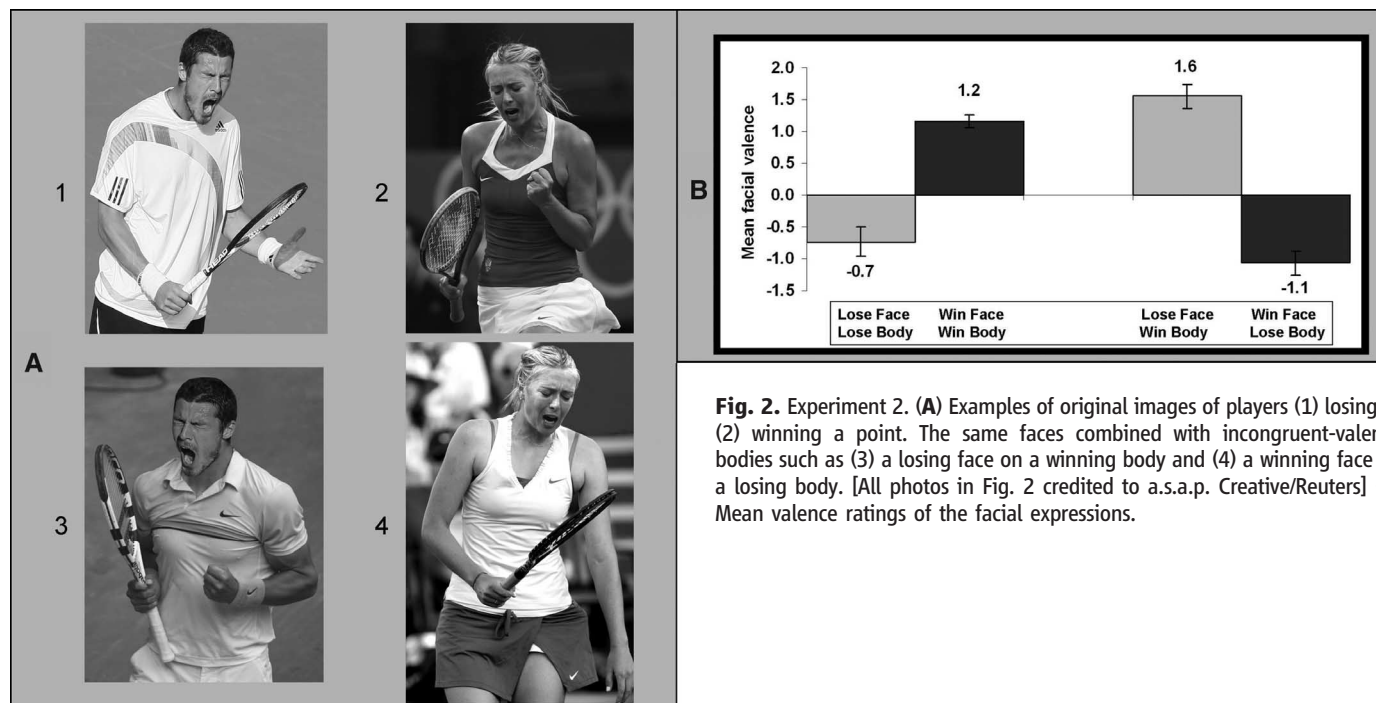


Fig. 2. Experiment 2. (A) Examples of original images of players (1) losing or (2) winning a point. The same faces combined with incongruent-valence bodies such as (3) a losing face on a winning body and (4) a winning face on a losing body. [All photos in Fig. 2 credited to a.s.a.p. Creative/Reuters] (B) Mean valence ratings of the facial expressions.

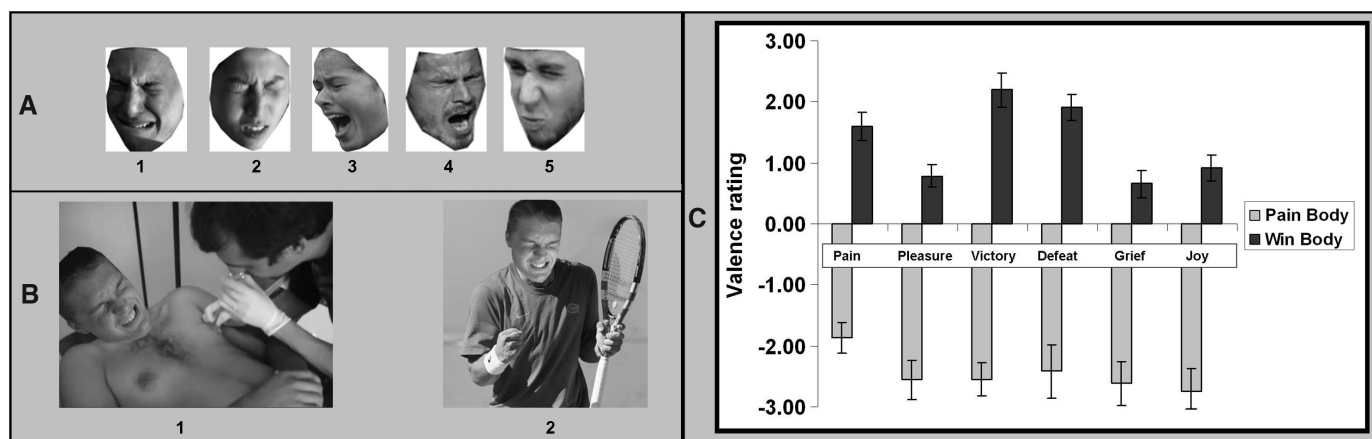


Fig. 3. Experiment 3. (A) Examples of isolated faces: 1 = grief, 2 = pleasure, 3 = victory, 4 = defeat, 5 = pain. Intense joy (not shown due to copyright reasons) appeared as a facial combination of grief and shock. [Photos 3A1, 3A3, 3A4 credited to a.s.a.p. Creative/Reuters. Photo 3A2 courtesy of beautifulagony.com. Photo 3A5 courtesy of Christopher Brown]

(B) Examples of contextualized facial expressions: (1) pleasure face with a painful body, and (2) the same face with a victorious body. Face expression in Photo 3B1 and 3B2 courtesy of beautifulagony.com. (C) Mean facial valence of the six facial categories in positive (winning) or negative (piercing) body context.

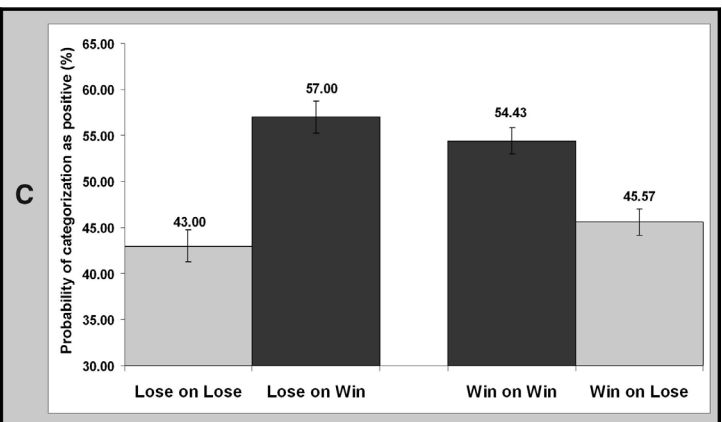
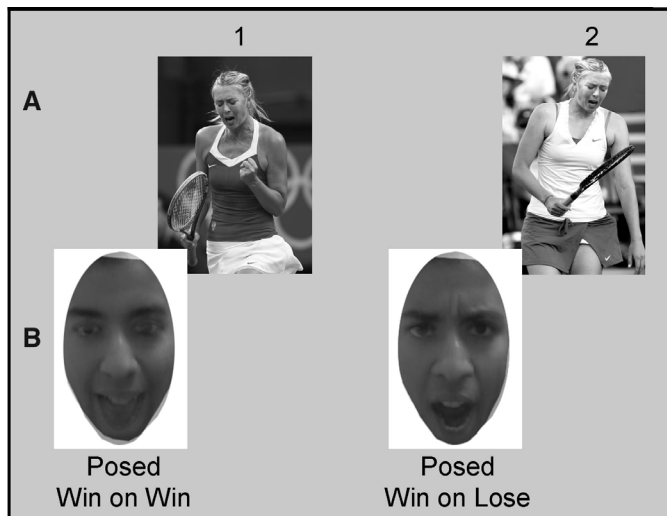


Fig. 4. Experiment 4. (A) Examples of contextualized facial expressions (1 = win on win, 2 = win on lose) viewed individually by “posing subjects.” [Photos in Fig. 4A1 and Fig. 4A2 credited to a.s.a.p. Creative/Reuters] (B) Illustrative visualization of posing behaviors, morphed across posers, shows characteristic

differences between the posing of an identical winning face on a winning versus losing body context. [Face visualizations in Fig. 4B from the Todorov lab, Princeton, NJ] (C) A new group of participants viewed pairs of poser faces (e.g., lose on lose versus lose on win) and chose the more positive face. Mean values represent the probability of rating the posed faces as conveying more positive valence.

debriefed participants were unaware of the manipulation. Posing participants viewed the images one at a time and adjusted their facial expressions to fit those in the face on the screen. Face movements were captured on video, and the resulting poses of the critical facial expression were converted from the video to still images.

In the “perception phase,” a new group of participants viewed the images of the posers’ headshots obtained in the first phase and categorized their affective valence. Specifically, participants simultaneously viewed two faces of an individual poser simulating the exact same face in different contexts. For example, picture 1 would show the face of a participant posing a winning face combined with a winning body, whereas picture 2 would show the face of a participant posing the same winning face combined with a losing body. Participants viewed the two posed faces and decided which expressed more positive valence.

As predicted, the results showed that the affective posing of identical faces shifted systematically as a function of the body’s affective valence [exact binomial test, P (two-tailed) < 0.006] (Fig. 4). Specifically, losing faces were posed as more positive when the poser viewed them on winning bodies than on losing bodies [exact binomial test, P (two-tailed) < 0.01]. Conversely, winning faces were posed as more negative when the poser viewed them on losing bodies than on winning bodies [exact binomial test, P (one-tailed) < 0.03]. Notably, posers had unlimited viewing of the contextualized faces, yet their motor simulation shifted as a function of the body’s affective valence. This suggests that the illusory facial affect previously reported reflects a genuine and automatic perceptual effect.

In sum, contrary to lay intuition and basic models of emotional expression and perception,

the studies presented here suggest that transient peak-intensity facial expressions elicited in a wide variety of emotional situations do not convey diagnostic information about affective valence. Paradoxically, although the faces are inherently ambiguous, viewers experience illusory affect and erroneously report perceiving diagnostic affective valence in the face. This process seems to be automatic as participants have little awareness of the actual facial ambiguity and the original diagnostic source of the valence (28, 29). In line with recent work on expression perception (30), the current data highlight the critical role of contextual body and scene information in the perception of facial affect (31–34) and confirm the elusive gap between artistic truth (the expressions people expect) and optical truth (the expressions that actually occur) (35, 36). Although previous work has shown that specific emotions are hard to recognize from weak and vague spontaneous expressions (37), such findings do not challenge standard models of emotions because these models can easily accommodate context effects on ambiguous emotional states. In contrast, the finding that peak face expressions of highly intense situations cannot be discriminated on the most basic dimension of positivity and negativity poses a major challenge to standard models of emotion.

We suggest two putative complementary explanations to account for the nondiagnosticity of intense faces. At the muscular level, the nondiagnosticity may reflect a transient signaling breakdown occurring because the facial musculature is not suited for accurately conveying extremely intense affect. Much like speakers blaring at maximum volume, the quality of the facial signal becomes degraded and noisy. At the affective level, the nondiagnosticity may also reflect an overlap in experience during high-intensity

emotions. Specifically, the overwhelming high intensity may move to the front of the stage of conscious experience, irrespective of the affective valence of the emotions (38). This transient degradation in signal quality need not be considered dysfunctional because (i) the ambiguity is rapidly resolved by contextual information and (ii) the face resumes diagnosticity shortly after the peak intensity resides.

Finally, although the current studies focused on the nondiagnosticity of intense facial expressions, future work may show that the underlying principles need not be limited to the visual facial modality. For example, peak emotional expressions in the auditory modality (consider intense vocal expressions of grief versus joy or pleasure versus pain, and so forth) may exhibit essentially the same patterns of nondiagnosticity as seen in faces.

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Supplementary Materials

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Fig. S1

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A Mutation in EGF Repeat-8 of Notch Discriminates Between Serrate/Jagged and Delta Family Ligands

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Notch signaling affects many developmental and cellular processes and has been implicated in congenital disorders, stroke, and numerous cancers. The Notch receptor binds its ligands Delta and Serrate and is able to discriminate between them in different contexts. However, the specific domains in Notch responsible for this selectivity are poorly defined. Through genetic screens in *Drosophila*, we isolated a mutation, *Notch^{jigsaw}*, that affects Serrate-but not Delta-dependent signaling. *Notch^{jigsaw}* carries a missense mutation in epidermal growth factor repeat-8 (EGF-8) and is defective in Serrate binding. A homologous point mutation in mammalian Notch2 also exhibits defects in signaling of a mammalian Serrate homolog, Jagged1. Hence, an evolutionarily conserved valine in EGF-8 is essential for ligand selectivity and provides a molecular handle to study numerous Notch-dependent signaling events.

The evolutionarily conserved Notch (N) signaling pathway affects numerous cell fate and differentiation events as well as proliferation and cell death (1). Signal activation is initiated by the binding of N receptor to ligands, Delta (Dl) or Serrate (Ser) (2). The majority of the extracellular domain of N receptor is composed of epidermal growth factor repeats (EGFs) (Fig. 1A). EGF-11 and EGF-12 are necessary for ligand-receptor interactions with both Dl and Ser (3), whereas EGF-24 to EGF-29 (Abruptex domain) negatively regulate these interactions (4). Although the *in vivo* role of most EGFs is unknown, mutations in these repeats are associated with numerous human diseases, including cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) (5), Alagille syndrome (ALGS) (6), aortic valve diseases (AVDs) (7–9), and squamous cell carcinoma (SCC) (10–12). Mammals have four N paralogs (*NOTCH1* to *NOTCH4*) that share sim-

ilar structural organizations, but only one N gene exists in *Drosophila*, simplifying structure-function analysis *in vivo* (table S1).

To obtain N mutations, we performed an F3 mosaic genetic screen on the X chromosome (fig. S1) and isolated 42 additional alleles of N. Twenty-one alleles carry different missense mutations and were grouped into eight distinct classes on the basis of molecular and phenotypic features (tables S2 and S3). All mutated residues are conserved in most human N paralogs.

One mutation, Valine361-to-Methionine (V361M) in EGF-8, *N^{jigsaw}*, exhibits defects in the wing margin without affecting venation or bristle development in mutant clones (Fig. 1, B to F, and table S3). Hemizygous mutants of *N^{jigsaw}* are pupal lethal, and *N^{jigsaw/+}* flies do not display wing notching. *N^{jigsaw}* fails to complement the lethality of null alleles of N and is rescued by a genomic rescue construct. Homozygous mutant clones in the wing exhibit strong notching and occasional

ectopic wing margin formation (Fig. 1, D and E). We did not observe any bristle density or cell fate defects (Fig. 1F and figs. S2 and S3). Hence, *N^{jigsaw}* displays a specific phenotype for a lethal N allele as inductive signaling is impaired, whereas lateral inhibition and lineage decisions remain unaffected.

Because the phenotypes associated with *N^{jigsaw}* are similar to Ser loss of function (13, 14), we determined whether N-Ser signaling is compromised. In the early wing primordium, N is activated at the boundary between the dorsal and ventral compartments (Fig. 1G). The dorsal domain expresses both Ser and Fringe (Fng) (15), whereas Dl is mainly expressed ventrally (16). Fng, a β -N-acetylglucosaminyltransferase that adds N-acetylglucosamine (GlcNAc) to O-fucosylated EGFs, modifies N so that it can be activated by Dl but not Ser (17, 18). As a result, Dl activates N in the dorsal domain, and Ser activates N in the ventral domain in cells flanking the dorsal-ventral boundary (Fig. 1, G and H). In *N^{jigsaw}* hemizygous discs, N activation is severely reduced or lost (Fig. 1I). Furthermore, cells that activate N signaling are present in the dorsal but not ventral compartment (Fig. 1I'). These data indicate that *N^{jigsaw}* is defective in N-Ser but not N-Dl signaling. This was confirmed in mosaic tissues (fig. S4). Last, *N^{jigsaw}* mutant clones can be ectopically activated by overexpression of Dl but not by Ser (fig. S5).

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The defects in N-Ser signaling in *N^{jigsaw}* mutants are not confined to the wing. Ser is required during hematopoiesis for crystal cell specification in the lymph glands (19). In *N^{jigsaw}* mutant animals, the crystal cell marker Lozenge is not expressed (fig. S6, A and B), resulting in a loss of circulating crystal cells (fig. S6, C to E). Furthermore, similar to *N^{jigsaw}* (fig. S6, F and G), loss of Ser causes a loss of salivary imaginal ring cells (20). Hence, multiple processes requiring N-Ser signaling are severely impaired in *N^{jigsaw}* mutants.

To determine the cause of ectopic wing margin formation in *N^{jigsaw}* mutant clones (Fig. 1E),

we monitored N activity. We observed ectopic N signal activation in wild-type (WT) cells adjacent to *N^{jigsaw}* mutant cells in the ventral compartment near the dorsal-ventral boundary (Fig. 2, A and B). Binding of N to Ser in cis mediates the removal of Ser from the plasma membrane in WT cells (ligand-cis-inhibition) (Fig. 2D) (21), whereas in mutant cells, Ser is up-regulated (Fig. 2C and fig. S7A). The ectopic activation of N in the neighboring WT cells can be suppressed by reducing the levels of Ser specifically in *N^{jigsaw}* mutant cells (fig. S7B), suggesting that *N^{jigsaw}* mutant cells are defective in ligand-cis-inhibition (Fig. 2E). Hence, *N^{jigsaw}* is defective in Ser-mediated

signaling, both in trans and in cis, whereas N-DI signaling appears unaffected.

Changes in the glycosylation status of EGFRs by Fng are known to modulate the ligand selectivity of Notch (17, 18). When one copy of *fng* is removed, notching in *N^{jigsaw}* clones is enhanced, whereas overexpression of Fng in *N^{jigsaw}* mutant clones partially suppresses notching (figs. S8 and S9). These data suggest that *N^{jigsaw}* and Fng affect the same process independently.

To test whether *N^{jigsaw}* affects the glycosylation status of N, we analyzed glycopeptides using mass spectrometry (22, 23). The *N^{jigsaw}* mutation does not cause a significant change in

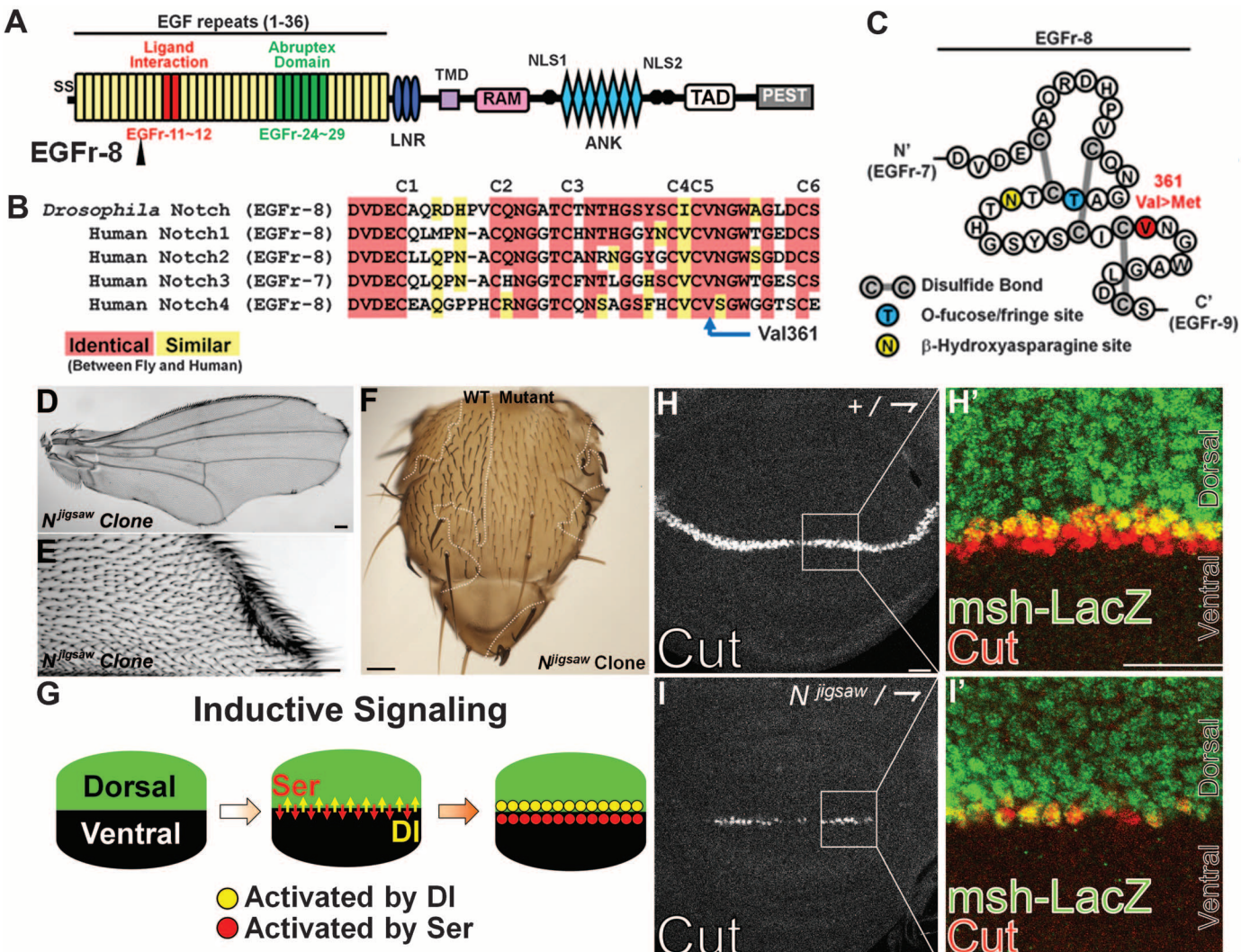


Fig. 1. *N^{jigsaw}*, a V361M mutation in a conserved domain in EGFr-8, is defective in Ser-mediated signaling. (A) Schematic representation of N. LNR, Lin12/Notch repeats; TMD, transmembrane domain; RAM, RBP-jk-associated molecule domain; NLS, nuclear localization signal; ANK, ankyrin repeat domain; TAD, transactivation domain; PEST, Pro-Glu-Ser-Thr rich domain. (B) Valine 361 mutated in *N^{jigsaw}* is conserved in Notch paralogs. (C) Schematic diagram of EGFr-8. [Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.] (D) Wings with *N^{jigsaw}* mutant clones exhibit notching. (E) Ectopic

wing margin in wings with *N^{jigsaw}* mutant clones. (F) *N^{jigsaw}* homozygous mutant bristles, marked by *yellow⁻*, are present in their normal stereotypic pattern. Homozygous WT bristles are *singed⁻*, and heterozygous bristles are *yellow⁺ singed⁺*. (G) Diagram of wing margin formation. (H to I') N activation at the dorsal-ventral boundary of WT and *N^{jigsaw}* mutant discs. [(H) and (I)] Low and [(H') and (I')] high magnification images of third instar wing imaginal discs of [(H) and (H')] WT and [(I) and (I')] *N^{jigsaw}* hemizygous mutant larva. The dorsal domain is labeled with β -galactosidase (LacZ) driven by the *msh* enhancer (*msh-lacZ*; green). N activation is shown by Cut (red). Scale bars, 100 μ m (D) to (F); 20 μ m (H) to (I').

Fig. 2. Ligand-cis-inhibition of Ser is impaired in *N^{jigsaw}* mutant cells. (**A** to **B'**) *N^{jigsaw}* clones in larval wing discs (marked by the absence of GFP, green). Cell-nonautonomous ectopic N activation is indicated by arrows [Wingless (Wg) in (**A**) and arrowheads [Cut in (**B**)]. Clones were induced by (**A**) *Ubx-FLP* or (**B**) *hs-FLP*. (**C** and **C'**) Expression of Ser (blue) is elevated in mutant clones, leading to cell-nonautonomous N activation (monitored by Cut) in surrounding WT neighboring cells. Scale bars, 20 μ m. (**D** and **E**) Schematic diagrams of ligand-cis-inhibition and cell-nonautonomous N activation. In *N^{jigsaw}* mutant cells, Ser accumulates at the cell surface and triggers signaling in the neighboring WT cells (**E**). WT cells are depicted in green, and *N^{jigsaw}* homozygous mutant cells are in gray.

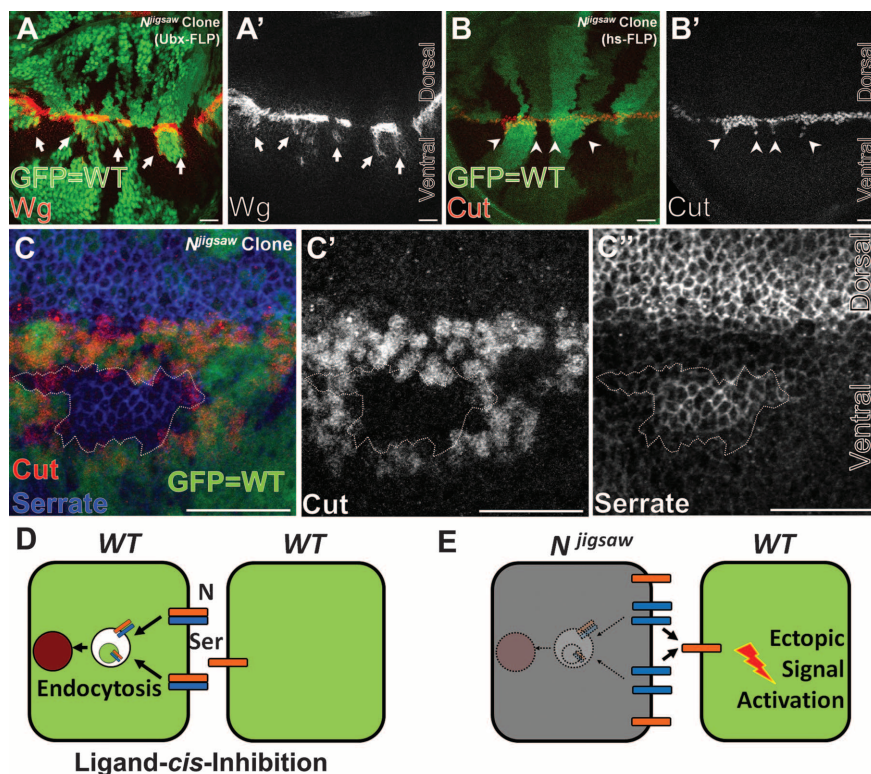
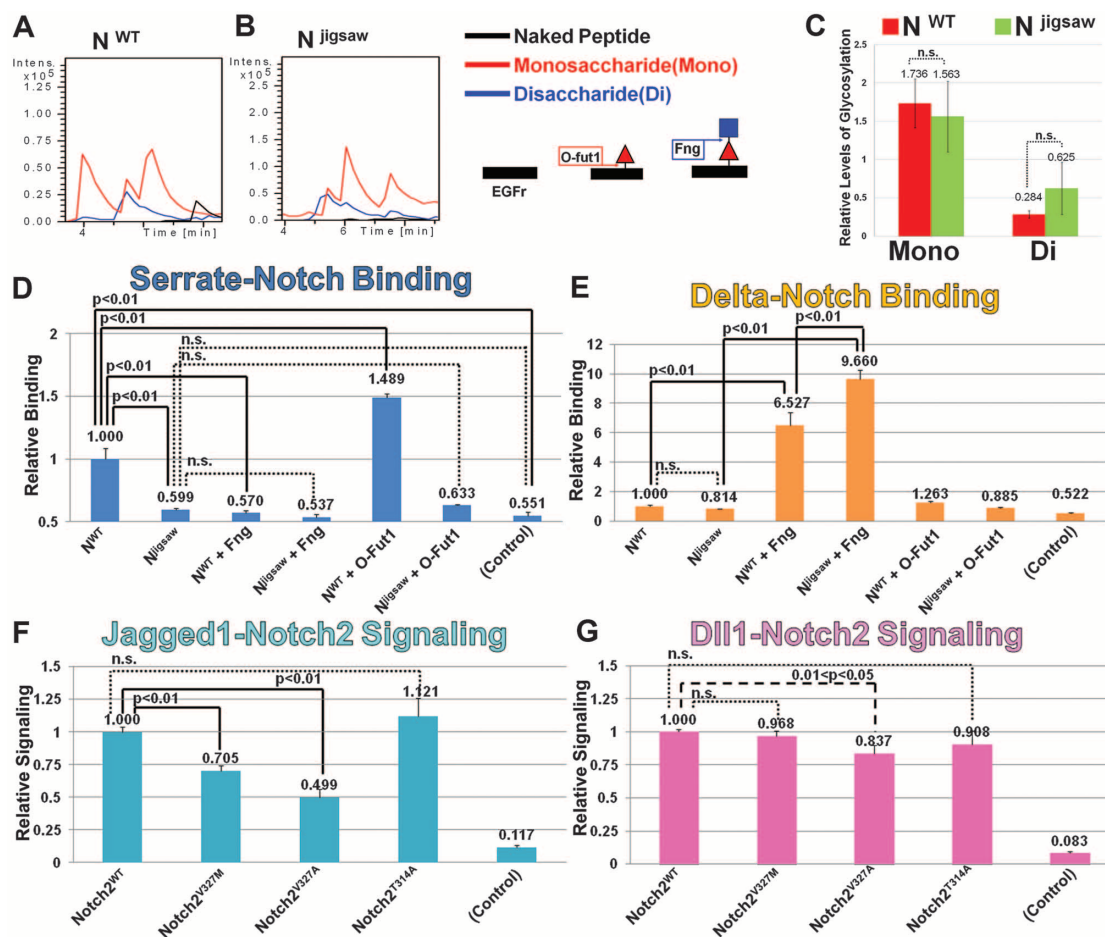


Fig. 3. *N^{jigsaw}* is defective in Ser binding in fly cells and Jagged1 signaling in mammalian cells, independent of Fng. (**A** and **B**) Multiple reaction monitoring (MRM) traces of ions show relative levels of unmodified (black line), *O*-fucose monosaccharide (red line), or *O*-fucose disaccharide (blue line) glycoforms of a peptide from EGFr-8 generated in the presence of Fng. Red triangle, fucose; blue square, GlcNAc; black rectangle, peptide. (**C**) Analysis of relative levels of monosaccharide versus disaccharide forms of the peptide from EGFr-8 based on the average of multiple MRM analyses (fig. S10, C and D). (**D** and **E**) Ligand-receptor binding assays in *Drosophila* S2 cells reveal loss of N-Ser binding in *N^{jigsaw}*. Binding of *N^{WT}* and *N^{jigsaw}* to Ser is shown in (**D**), whereas binding of *N^{WT}* and *N^{jigsaw}* to DI is shown in (**E**). (**F** and **G**) Mammalian cell-based assays show that V327M in mouse Notch2 reduces receptor activation by Jagged1 (**F**) but not Dll1 (**G**). The V327A reduces Notch2-Jagged1 signaling even more (**F**), whereas a mutation in the *O*-fut1/Fng modification site of EGFr-8 (T314A) does not affect Jagged1 or Dll1 signaling [(**F**) and (**G**)]. Error bars indicate SEM.



the level of *O*-fucosylation and Fng-mediated elongation of *O*-fucose on EGFr-8 (Fig. 3, A to C, and fig. S10). In addition, other EGFRs do not display alterations in *O*-fucosylation and Fng-mediated elongation (fig. S11), indicating that these EGFRs are properly folded, which is a requirement for glycosylation. Hence, the *N^{jigsaw}* mutation has little or no effect on the sugar modification status of N, again suggesting that the defects in N-Ser signaling are independent of Fng.

Because both trans- and cis-signaling of N-Ser are defective in *N^{jigsaw}* mutant cells, and the expression, exocytosis, and endocytosis of *N^{jigsaw}* are indistinguishable from *N^{WT}* (figs. S12 and 13), we tested whether *N^{jigsaw}* affects Ser binding (17, 24). In contrast to *N^{WT}*, *N^{jigsaw}*-Ser binding is significantly diminished, and this interaction is unaffected by the presence of Fng (Fig. 3D). Moreover, overexpression of O-fut1, a sugar-modifying enzyme and molecular chaperone that can strengthen *N^{WT}*-Ser binding (24), cannot improve *N^{jigsaw}*-Ser binding (Fig. 3D). In contrast, *N^{jigsaw}* interacts with DI in the presence of Fng (Fig. 3E). These data indicate that *N^{jigsaw}* directly impairs N-Ser binding without disrupting N-DI binding.

To assess whether *N^{jigsaw}* (V361M) plays a similar role for a mammalian N, we performed a signaling assay (25). We introduced V327M (table S2) into mouse Notch2 and tested its ability to respond to Jagged1 or Delta-like1 (Dll1). Notch2-Jagged1 signaling is severely decreased, whereas Notch2-Dll1 remains unaffected (Fig. 3, F and G). In addition, a V327A mutation causes a further decrease in Notch2-Jagged1 signaling, showing that loss of valine is responsible for the *N^{jigsaw}* phenotype. In contrast, a mutation in the conserved O-fut1/Fng-modification site within Notch2 EGFr-8 (T314A) does not affect Jagged1- or Dll1-mediated signaling.

Among the identified *N* mutations (tables S2 and S3) and other reported *N* alleles (26, 27), *N^{jigsaw}* separates N-Ser from N-DI binding and signaling in vivo. Previously, two *N* alleles have

been shown to exhibit wing margin defects only when mutant cells are in the ventral compartment, suggesting defective Ser-mediated signaling (28). However, these mutations do not affect the EGFRs, and the nature of the signaling defects remains uninvestigated. Our data suggest that in addition to EGFr-11 and EGFr-12, which are required for both DI and Ser binding (3), EGFr-8 plays a critical role in N-Ser interactions. The *N^{jigsaw}* mutation does not obviously affect the trafficking or glycosylation of N. Therefore, EGFr-8 may directly be involved in ligand-receptor interactions together with EGFr-11 and EGFr-12 or may be crucial for N to adopt a conformation favoring Ser binding.

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Supplementary Materials

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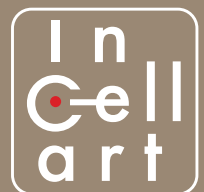


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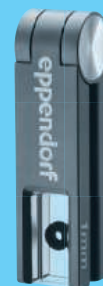
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Two new products have been designed to detect primary antibodies of mouse (WB-2000) or rabbit (WB-1000) origin in Western blot detection: WestVision Peroxidase Polymer Anti-Rabbit IgG and WestVision Peroxidase Polymer Anti-Mouse IgG. The WestVision conjugates are based on a new method of polymerizing enzymes and attaching these polymers to antibodies, allowing a higher density of enzymes to access a target with minimal interference. The result is an excellent signal-to-noise ratio in Western blot detection. WestVision reagents are also economically priced, allowing high sensitivity, low background, and lower cost in Western blot detection. With a chromogenic substrate, the reagents provide enough working solution to typically detect 20 to 200 blots (10 x 10 cm). With a chemiluminescent substrate, dependent on the dilution employed, 800 to 8,000 blots can be detected.

Vector Laboratories

For info: 800-227-6666 | www.vectorlabs.com

CAMKII ANTIBODY

A new antibody is available to detect the oxidized form of calmodulin-dependent protein kinase II (CaMKII), which can be used to characterize and diagnose various diseases. The activation of CaMKII through oxidation is associated with a variety of conditions including heart failure, cardiac arrhythmias, cancer, neurological disease, and sepsis. CaMKII oxidation increases during myocardial ischemia and infarction, and CaMKII oxidation has been shown to play a pivotal role in cardiac cell death during angiotensin II-mediated apoptosis. While previous methods of measuring oxidative stress have relied on "bystander" molecules that are not directly implicated in disease pathogenesis, GeneTex's new antibody against oxidized CaMKII will allow researchers to track the oxidation of a biologically active molecule present in blood or biopsy specimens.

GeneTex

For info: 877-436-3839 | www.genetex.com

HIS-TAG PURIFICATION RESIN

For all scientists requiring highly purified protein, Roche's cOMplete His-Tag Purification Resin is a high-capacity immobilized metal ion affinity chromatography (IMAC) matrix for one-step purification of His-tagged proteins from lysates. In contrast to currently available nitrilotriacetic acid (NTA) resins or resins using chelators, such as iminodiacetic acid (IDA), the nickel ions in this resin are immobilized using one of the strongest chelators known. This new proprietary chemistry minimizes nickel ion leakage, even when using EDTA and DTT containing buffers. This powerful feature, in addition to eliminating the need for resin nickel recharging, also stabilizes purified proteins by preventing nickel ions from catalyzing protein oxidation.

Roche

For info: 800-428-5433 | www.roche.com

KINASE ASSAYS

Three new kinase assays, all based on the powerful HTRF technology, were designed to provide new, easy, and rapid tools for the study of different signaling pathways in drug discovery research, from high throughput screening to lead optimization phases. The assays, Phospho-STAT3 (Signal Transducer and Activator of Transcription 3) (Tyr705), Phospho-p38 MAPK (Thr180/Tyr182), and Phospho-IKK β (Ser177/Ser181) involve targets that are associated with many diseases, including cancer, rheumatoid arthritis, Crohn's disease, pulmonary fibrosis, and acute lung injury. The three new cellular kinase assays are homogeneous, nonradioactive, and nonbead based. Their mix and read protocol eliminates the need for washing steps and their long signal stability and flexibility allow for multiple experiments—including small series—without requiring special equipment, saving valuable time and cost.

Cisbio Bioassays

For info: 888-963-4567 | www.cisbio.com

Electronically submit your new product description or product literature information! Go to www.sciencemag.org/products/newproducts.dtl for more information.

Newly offered instrumentation, apparatus, and laboratory materials of interest to researchers in all disciplines in academic, industrial, and governmental organizations are featured in this space. Emphasis is given to purpose, chief characteristics, and availability of products and materials. Endorsement by *Science* or AAAS of any products or materials mentioned is not implied. Additional information may be obtained from the manufacturer or supplier.



Announcing our new partnership with NASA Federal Credit Union

Dear Member:

AAAS is committed to offering you member benefits that fit your needs and make your membership more valuable.

With that in mind AAAS is embarking on a new partnership with NASA Federal Credit Union that will provide you with access to a wide range of financial tools and products. Like AAAS, NASA Federal Credit Union is dedicated to serving the scientific community. This shared perspective is just one of the many reasons that we are embarking on this partnership.

As you may know, we have recently ended our banking relationship with Bank of America, but we're confident that our new partnership with NASA FCU will provide you with a superior banking experience. NASA FCU offers members better ways to save and smarter ways to borrow with friendly, professional service – along with anytime, anywhere account access.

Moreover, unlike other financial institutions that have public stockholders, NASA FCU is a not-for-profit financial cooperative where being a member means being an owner, too. And as a member/owner, you will enjoy unique benefits like: better loan rates, higher dividends and state-of-the-art products and services.

We'll be sending you more information about this great new benefit over the coming months. In the meantime, be sure to visit nasafcu.com/AAASpackage to apply for the new AAAS Platinum Advantage Rewards or Platinum Cash Rewards credit cards. You can also take a sneak peek at the AAAS Check Card and Checks coming soon.

Sincerely,

A handwritten signature in blue ink, appearing to read 'Ian King'.

Ian King
Director of Marketing and Membership, AAAS



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Science Careers

From the journal *Science*



ETH

Eidgenössische Technische Hochschule Zürich
Swiss Federal Institute of Technology Zurich

Professor of Physical Chemistry

The Laboratory of Physical Chemistry of the Department of Chemistry and Applied Biosciences at ETH Zurich (www.chab.ethz.ch) invites applications for the above-mentioned position.

The applicant's research field should preferably focus on chemical kinetics and intra-molecular dynamics with a clear emphasis on the development and applications of new experimental methods. The combination of experiment and theory is considered important. Responsibilities include teaching in all areas of physical chemistry. The new professor will be expected to teach undergraduate level courses (German or English) as well as graduate level courses (English).

Your application should include your curriculum vitae and list of publications. The letter of application should be addressed to the **President of ETH Zurich, Prof. Dr. Ralph Eichler**. The closing date for applications is **28 February 2013**. ETH Zurich is an equal opportunity and affirmative action employer. In order to increase the number of women in leading academic positions, we specifically encourage women to apply. ETH Zurich is further responsive to the needs of dual career couples and qualifies as a family friendly employer. Please apply online at www.facultyaffairs.ethz.ch.

BLUE WATERS

Tackling Some of Society's Biggest Scientific Challenges? Think Illinois and the Blue Waters Supercomputer.

The University of Illinois at Urbana-Champaign's National Center for Supercomputing Applications is launching Blue Waters, one of the world's most powerful supercomputers with a peak performance of more than 11.5 petaflops. Building on more than a half-century of high-performance computing leadership at Illinois, Blue Waters is supported by the National Science Foundation.

Scientists around the country will use Blue Waters to tackle some of society's biggest challenges—understanding the building blocks of the universe, predictive geophysics, fundamentals of combustion, energy conversion and distribution, nanotechnology for electronics and medical devices, biomedical imaging and

biophysical modeling, and the discovery and design of materials.

Part of Blue Waters could be yours. The University of Illinois has openings for several Blue Waters Professors, available to prospective faculty at any rank, who will receive substantial allocations on the supercomputer and expedited access to the system.

As part of the Visioning Future Excellence at Illinois initiative, Blue Waters faculty appointments will be made to individual departments within the College of Engineering.

**For details on how to apply, go to:
engineering.illinois.edu/bluwater**

Illinois is an Affirmative Action/Equal Opportunity Employer and welcomes individuals with diverse backgrounds, experiences, and ideas who embrace and value diversity and inclusivity. (www.inclusiveillinois.illinois.edu)

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FACULTY POSITION

Math

NYU SHANGHAI

NYU Shanghai is currently inviting applications for a non-tenure teaching professor in Mathematics. We seek candidates with experience in teaching a variety of subjects including calculus, lineal algebra, and differential equations. The search will remain open until the position is filled, but review of applications will begin January 15, 2012.

NYU Shanghai is the newest degree-granting campus within NYU's global network. It is the first Sino-US higher education joint venture to grant a degree that is accredited in the US as well as in China. A research university with liberal arts and sciences at its core, it resides in one of the world's great cities that is also a vibrant intellectual community. NYU Shanghai will recruit scholars who are committed to our global vision of transformative teaching and innovative research.

New York University has established itself as a Global Network University, a multi-site, organically connected network encompassing key global cities and idea capitals. The network has three degree-granting campuses - New York, Shanghai, and Abu Dhabi - complemented by 12 additional academic centers across five continents. Faculty and students circulate within the network in pursuit of common research interests and cross-cultural, interdisciplinary endeavors, both local and global.

The terms of employment are comparable to U.S. institutions. The appointment might begin as soon as August 1, 2013, or could be delayed until August 1, 2014.

Applicants should submit curriculum vitae, statement of research and teaching interests, representative publications if applicable, and three letters of reference in PDF format to be considered. Please visit our website at www.nyuopsearch.com/applicants/Central?quickFind=51493 for instructions and other information on how to apply. If you have any questions, please e-mail shanghai.faculty.recruitment@nyu.edu.



NYU Shanghai is an Equal Opportunity/Affirmative Action Employer.

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LOUISVILLE

BIRTH DEFECTS CENTER

[HTTP://LOUISVILLE.EDU/BIRTHDEFECTSCENTER/](http://LOUISVILLE.EDU/BIRTHDEFECTSCENTER/)

Tenure-Track Faculty Position Molecular Determinants of Craniofacial Development

The University of Louisville Birth Defects Center, a NIH-funded Center for Biomedical Research Excellence, and the Department of Molecular, Cellular & Craniofacial Biology in the School of Dentistry, invite applications for a tenured or tenure-track faculty appointment at the level of Assistant, Associate or Full Professor. Candidates should have an active research program in the area of molecular and/or molecular genetic aspects of craniofacial development, which also complements other existing strengths in the Center in epigenetic determinants of normal and abnormal embryonic development, and developmental origins of adult disease. Candidates must have a Ph.D. degree, or the equivalent, in a biomedically related field, and a sound research program reflected by a robust publication record and a strong history of extramural funding. Successful candidates will be expected to maintain an independent and innovative research program that attracts and sustains extramural funding.

Applicants must apply online at: www.louisville.edu/jobs for position #27592, at which time a *curriculum vitae* must be uploaded. In addition, applicants should send a single pdf document containing: (1) a letter of intent, (2) a statement of current research activities and plans, and (3) names and contact information for three references to: **Dr. Bob Greene; Chair, Department of Molecular, Cellular & Craniofacial Biology; Director, Birth Defects Center**, e-mail: Dr.Bob.Greene@gmail.com.

*The University of Louisville is an EEO/AA Employer.
Women and minorities are encouraged to apply.*

SANFORD
HEALTH

SCIENTIST/SENIOR SCIENTIST POSITION CANCER BIOLOGY RESEARCH CENTER SANFORD RESEARCH/USD

The Cancer Biology Research Center (CBRC) invites applications from researchers for a full time faculty position at the rank of Associate Scientist or Scientist within Sanford Research/USD, the research division of Sanford Health in Sioux Falls, South Dakota. A historic \$400 million gift by philanthropist Denny Sanford has allowed for expansion of Sanford Research/USD with the opening of the new Sanford Research Center (300,000 sq ft) and the establishment of a collaboration with the Sanford-Burnham Institute for Medical Research La Jolla. These provide researchers with state of the art core facilities and are enabling the creation of an integrated, world class, academic research network.

We seek an outstanding basic, translational, or clinical research scientist with an active research program in Cancer Biology. Successful candidates will have research interests that complement ongoing research programs and are compatible with long term goals of the institution. Applicants must hold a PhD, DVM, MD or MD/PhD degree. Candidates must have strong publication records. Appointments at the Scientist level are expected to have a demonstrated track record of extramural grant support. Successful candidates will join the energetic and collegial research community at Sanford Research/USD. Please see <http://www.sanfordresearch.org/> for further information.

Significant institutional support including modern laboratory space and state-of-the-art facilities will be provided in the new Sanford Research Center. In addition, a comprehensive compensation package will be tailored to the individual's qualifications. Sanford Health is an Equal Opportunity/Affirmative Action Employer. Candidates should submit a detailed curriculum vitae, description of research experience and future plans, and at least three letters of recommendation. Application materials should be sent to:

W. Keith Miskimins, PhD

Director, Cancer Biology Research Center

Sanford Research/USD

2301 East 60th Street North, Sioux Falls, SD 57117-5039

Telephone: 605-312-6104 FAX: 605-312-6071

Email: keith.miskimins@sanfordhealth.org



The University of New Mexico

Landscape Ecologist The University of New Mexico Department of Biology

The Department of Biology at the University of New Mexico invites applications for a full-time, tenure-track probationary appointment at the Assistant Professor level, beginning in Fall 2013.

Minimum Qualifications include a Ph.D. and post-doctoral experience by August 15, 2013. For complete details or to apply, please visit: <https://unmjobs.unm.edu/> and reference the posting number **0818385**. Best consideration date is January 22, 2013. Review of applications will commence on **January 22, 2013**. The position will remain open until filled.

Preferred Qualifications: The successful candidate will demonstrate excellence in research as evidenced by pre and post-doctoral work; have a demonstrated publication record in peer-reviewed journals; be committed to establishing an internationally recognized and externally funded research program in the area of landscape ecology. Preference is given to areas of research that complement existing strengths in the Department of Biology; to candidates that demonstrate a commitment to excellence in teaching at the undergraduate through graduate level in a minority majority institution; and to applicants that show enthusiasm for working in a broad biology department with diverse research strengths.

To apply, applicants must submit a letter of interest, curriculum vitae, three recent publications, statements of research and teaching interests, and a list of five references. All materials must be submitted directly to <https://unmjobs.unm.edu/> by the best consideration date. When letters of recommendation are requested, the letters should be emailed as PDF attachments to LSEASST@unm.edu as soon as possible.

Questions can be directed to **Dr. Cliff Dahm** (cdahm@sevilleta.unm.edu).

The University of New Mexico is an Equal Opportunity/Affirmative Action Employer and Educator. Women and underrepresented minorities are encouraged to apply.



Chair, Department of Biochemistry

Northwestern University Feinberg School of Medicine invites applications and nominations for the position of Chair, Department of Biochemistry.

Assuming leadership at the Feinberg School of Medicine, the Chair will join Feinberg at a crucial point in time with the formation of our national brand called Northwestern Medicine. The Chair is not just the department leader but also an important figure across the academic medical center and will build upon existing institutional strengths to lead the formation of new Department of Biochemistry with space and opportunity to recruit substantial numbers of new faculty.

Principal investigators appointed through the Feinberg School of Medicine, ranked 18th in *U.S. News & World Report*, are supported by \$371 million of annual research funding. The Chair will report directly to the Dean of the medical school. Successful candidates will possess a PhD or equivalent degree, be eligible for appointment as a full-time Professor, and have a record of scholarly accomplishments and national recognition in biochemistry.

Please email nominations and CVs of appropriate candidates to:

Patricia Spear, PhD
p-spear@northwestern.edu, chair of the search committee

and copy Ila Allen
ila@northwestern.edu, recruitment coordinator, Feinberg School of Medicine.

Applications will be taken until the position is filled. Northwestern University is an Affirmative Action, Equal Opportunity Employer. Women and minorities are encouraged to apply. Hiring is contingent upon eligibility to work in the United States.



THE UNIVERSITY OF TEXAS MDAnderson Cancer Center

Making Cancer History™

The Department of Molecular Carcinogenesis at Science Park seeks applications for a full-time faculty member at the rank of assistant professor, term tenure track. Research in the department focuses on cellular, molecular and genetic mechanisms of carcinogenesis. We seek individuals working in the areas of genetics, epigenetics, genomic instability, cancer stem cells and bioinformatics/genomics.

Candidates must be committed to working in a highly collaborative, interdisciplinary environment. The successful candidate will be expected to develop and maintain an internationally recognized and competitively funded research program, and to participate in graduate training. Required qualifications include a Ph.D. (or equivalent), postdoctoral or independent scholarly research experience, and a strong publication record.

The department is well funded with many outstanding core support services, including facilities for analysis of genetically engineered mouse models and next-generation sequencing. Our division is located in a pine-oak forest near the city of Austin, TX. The University of Texas MD Anderson Cancer Center offers outstanding research facilities, startup packages and faculty benefits. Refer to the Science Park Department of Molecular Carcinogenesis Web page for additional information.

Interested applicants should submit a statement of research interest, curriculum vitae and letters of reference by January 1, 2013 to the following email address: sciencepark@mdanderson.org.

MD Anderson Cancer Center is an equal opportunity employer and does not discriminate on the basis of race, color, national origin, gender, sexual orientation, age, religion, disability or veteran status except where such distinction is required by law. All positions at The University of Texas MD Anderson Cancer Center are security sensitive and subject to examination of criminal history record information. Smoke-free and drug-free facility.

Assistant Professor, Term Tenure Track

Sharon Y.R. Dent, Ph.D.
 Professor and Chair
 Department of Molecular Carcinogenesis
 1808 Park Road 1C
 P.O. Box 389
 Smithville, TX 78957



VICE PRESIDENT FOR RESEARCH AND ECONOMIC DEVELOPMENT

The University of New Mexico (UNM), New Mexico's Flagship Research University, invites nominations and applications for the position of Vice President for Research and Economic Development (VPRED). This position is an excellent opportunity for a visionary leader to harness the scientific and creative potential of a diverse and highly motivated faculty. The VPRED also plays a vital role in capitalizing on the intellectual wealth and economic impact of the UNM Main/Branch research enterprise, using it as a driver for the overall enhancement of the institution and the state. Reporting to the Provost and Executive Vice President for Academic Affairs, the VPRED is the chief promoter of creativity and innovation and facilitator of the University's research mission. In addition, the VPRED is responsible for assuring the effective administration of research at all stages, ensuring its integrity and compliance with international standards of excellence.

The VPRED is directly responsible for an annual operating budget of approximately \$20 million and the administration of the Office of the Vice President for Research and Economic Development, Main Campus Pre-Award, Research Administration and Compliance, and direct-report centers, institutes, and programs. The VPRED is a member of the senior UNM leadership team and guides institutional strategic planning for research and sponsored program development. The VPRED is responsible for engaging campus constituencies in multidisciplinary research opportunities and the development of campus-wide initiatives to enhance one of UNM's roles as a driver of economic development in the State of New Mexico.

For best consideration submit application by **January 7, 2013**. The full job description and instructions on how to apply can be found at unmjobs.unm.edu/applicants/Central?quickFind=70125. Applications are to be submitted online at UNMJOBS. All nominations should be sent electronically to <https://docs.google.com/spreadsheets/viewform?formkey=dDZ6ZHRRXY2alhXSFfUb0Zvb1JSSnc6MQ>.

All qualified applicants are encouraged to apply, including women, minorities and those from other underrepresented groups. The University of New Mexico is an Equal Opportunity/Affirmative Action employer and educator.



The University of Georgia

(1) The Institute of Bioinformatics and the Franklin College of Arts and Sciences invite applications for a joint tenure-track faculty position in **bioinformatics or computational biology** at the assistant professor level, although exceptional candidates could possibly be considered at the early associate professor level. The candidate should have a Ph.D. or equivalent degree in the sciences and a strong research record at the interface of computing and life science. The successful candidate will join our highly-active interdisciplinary program in Bioinformatics (<http://job.uga.edu>) with a tenure home in one of the following Franklin College Departments: Cellular Biology, Computer Science, Genetics, Marine Sciences, Mathematics, Microbiology, Plant Biology, or Statistics (<http://www.franklin.uga.edu/academics/departments.php>). The candidate will be expected to maintain a rigorous, externally funded research program and contribute to undergraduate and graduate teaching. Applications must be uploaded as PDF documents to <https://www.franklin.uga.edu/jobs/bioinformatics> and should include a cover letter, CV, and brief statements of research and teaching interests. Three letters of recommendation should be uploaded separately to the same web site. The committee will begin reviewing applications on **December 6, 2012** and continue until the position is filled.

(2) The Department of Plant Biology (<http://www.plantbio.uga.edu>) invites applications for a tenure-track faculty position at the assistant or associate professor level in **plant ecology** with a focus on aboveground components. Areas of specialization can include, but are not limited to, population dynamics and plant interactions with their environment, other plants and organisms. This new position will have available research funds from a Haines Family Professorship endowment. We seek an individual who addresses fundamental ecological and evolutionary questions and who integrates multiple tools and techniques, potentially including field, lab, modeling, molecular, and genomic approaches. The successful candidate will have a Ph.D. degree in plant biology or any related field of study and a strong record of scientific productivity. S/he is expected to develop or expand a vigorous externally-funded research program and to teach and train undergraduate and graduate students. The Plant Biology Department encompasses a broad range of disciplines and has historical strengths in plant ecology, evolutionary biology and mycology. To apply, candidates should submit a cover letter, curriculum vitae, and short statements of research interests and teaching philosophy as a single PDF file to <https://www.franklin.uga.edu/jobs/plant-biology>. Three letters of recommendation should be uploaded separately to the same web site. The committee will begin reviewing applications on **January 7, 2013** and continue until the position is filled.

The Institute of Bioinformatics, Department of Plant Biology, Franklin College of Arts and Sciences, and University of Georgia are committed to increasing the diversity of their faculty and students, and to sustaining a work and learning environment that is inclusive. Women, minorities and people with disabilities are encouraged to apply. The University is an EEO/AA institution. Georgia is well known for its quality of life in regard to both outdoor and urban activities. UGA is a land- and sea-grant institution located in Athens, 90 miles northeast of Atlanta, the state capital (www.visitathensga.com; www.uga.edu).



Department Chair Position

The University of Idaho is seeking an outstanding leader for the Department of Biological Sciences. The Department of Biological Sciences consists of 25 faculty with dynamic extramurally funded research programs, with particular strengths in evolutionary biology, reproductive biology, cellular and molecular biology and biochemistry. This position represents an opportunity to lead new initiatives and guide the future growth of the department. This is an academic year, tenured position at the rank of professor. Qualified candidates must possess: a) a doctorate in biology or a related field; b) a professional record sufficient to achieve tenure at a senior rank; c) an internationally recognized, externally funded research program and; d) experience in teaching at the undergraduate and graduate levels. Outstanding candidates will also possess the ability to: a) lead large scale research initiatives; b) bridge academic disciplines and; c) work and communicate effectively with diverse groups including students, faculty, alumni, and university and state administrators. Review of applications begins 1/16/2013. For a full description and application materials, visit <http://apptkr.com/297645>. For questions relating to the application process, contact Gina Tingley at biofac@uidaho.edu.

EOE



Tenure Track Positions in Cardiovascular Biology

The Aab Cardiovascular Research Institute at the University of Rochester School of Medicine and Dentistry is seeking funded Assistant and Associate Professors to fill Associate and Full Professor tenured track positions. Generous recruitment packages are available. Outstanding scientists in all areas of cardiovascular sciences are encouraged to apply. We particularly encourage applications from investigators with accomplishments in the following areas: Atherosclerosis, Vascular Inflammation, Thrombosis, and Heart Failure.

The Aab CVRI offers competitive startup packages, excellent core facilities, and a highly interactive group of scientists: <http://www.urmc.rochester.edu/cvri/>

Interested applicants should submit their curriculum vitae, a one page summary of prior research accomplishments, a one page description of future research plans, and three letters of reference the following address: CVRIrecruiting@urmc.rochester.edu

The University of Rochester School of Medicine and Dentistry is an Equal Opportunity Employer.



ROSALIND FRANKLIN UNIVERSITY
OF MEDICINE AND SCIENCE

Advocate
Lutheran General Hospital

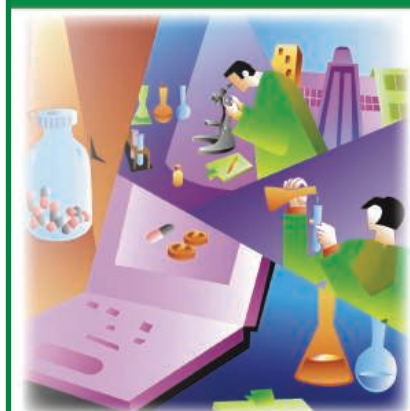
Inspiring medicine. Changing lives.

Vice Dean for Translational Research (RFUMS) Medical Director of the Russell Institute for Research and Innovation (RIRI) at Advocate Lutheran General Hospital (ALGH)

The Chicago Medical School at Rosalind Franklin University of Medicine and Science (RFUMS) and Advocate Lutheran General Hospital (ALGH) invite applications for the full-time position of Vice Dean for Translational Research (RFUMS) and Medical Director of the Russell Institute for Research and Innovation (RIRI) at Advocate Lutheran General Hospital (ALGH).

The Vice Dean for Translational Research Director of the Russell Foundation Research Institute will:
•Possess an MD or MD/PhD degree •Have extensive clinical and translational research experience and capabilities •Be expected to either currently maintain or have previously maintained a long-standing active research program with extramural funding •Have a broad research perspective with appreciation and knowledge across wide ranges of biomedical research •Possess the ability to bridge the gap between basic science, translational, and clinical research. Interested applicants should submit, electronically, a cover letter, curriculum vitae, including teaching and research interests and three names of reference to Rosalind Franklin University of Medicine and Science Human Resources web site via <http://rosalindfranklin.peopleadmin.com/postings/3395>. Applications will be accepted until **January 31, 2013**.

CAREER TRENDS Running Your Lab



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Brought to you by the
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Quantitative Biology Cluster Hire
University of Maryland, College Park
 College of Computer, Mathematical and Natural Sciences
 College of Agriculture and Natural Resources
 A. James Clark School of Engineering
 School of Public Policy

The University of Maryland, College Park seeks multiple tenured/tenure track faculty in the area of Quantitative Biology. The University counts among its researchers leaders at the interface of biological and physical sciences, and includes historically strong, world-recognized research programs on non-linear dynamics and statistical thermodynamics. With this initiative, the campus plans to significantly expand this expertise to create an outstanding interdisciplinary program harnessing the modern tools of biology, physics and engineering. We invite exceptional scientists with strong backgrounds in biology, physics, mathematics, technology development and/or entrepreneurship, whose research is advancing the frontiers of knowledge and contributing to a quantitative and integrated understanding of life.

The appointments may be made at the Assistant, Associate or Full Professor level, commensurate with qualifications and experience. Outstanding research facilities and a generous start-up package will be provided. The successful candidates will be expected to maintain cutting-edge externally funded research programs that synergize with existing core groups on campus, and to participate in undergraduate and graduate teaching. Applicants must have a doctorate degree, an outstanding publication record and a commitment to excellence in teaching.

The University of Maryland, College Park is the flagship campus of the University System of Maryland and one of the most rapidly advancing public research universities in the country. The campus is a highly interactive environment including various Centers and Institutes, such as those formed through partnership and collaboration with the National Institutes for Standards and Technology (NIST), the U.S. Food and Drug Administration (FDA) and the National Institutes of Health (NIH). Close proximity to Washington DC, Baltimore and the Maryland Biotechnology Corridor facilitates interactions with an extraordinary range of major research institutions such as NIST, NIH, NSF, DOE, NASA, FDA, USDA, NRL, ARL and HHMI-Janelia Farm, in addition to providing a rich cultural environment.

Applicants should apply electronically through Jobs@UMD at the following URL: <http://jobs.umd.edu/applicants/Central?quickFind=57836>

Applicants should submit the following: (1) cover letter, (2) curriculum vitae, (3) summary of research plans (maximum two pages), (4) one-page teaching philosophy statement and (5) the contact information with email of at least three references. Complete applications should be received by February 15, 2013, but will be accepted until the positions are filled.

*The University of Maryland is an Affirmative Action/Equal Opportunity Employer.
 Women and members of underrepresented groups are especially encouraged to apply.*



**Indian Institute of Science,
 Bangalore.**

Molecular Biophysics Unit
 (<http://mbu.iisc.ernet.in/>)

**Openings for Assistant Professor
 Positions.**

Applicants, preferably below 35 years, should have a Ph.D. with postdoctoral experience coupled with an excellent publication record.

We seek candidates in the general area of **structural and computational biology with an emphasis on understanding macromolecular systems at the molecular level.**

Applications with a detailed CV, research plan (not exceeding 3 pages) and names of at least 4 referees should be sent to chairman@mbu.iisc.ernet.in



**JOINT FACULTY POSITION
 BIOENGINEERING AND
 MATERIALS SCIENCE & ENGINEERING**

The Departments of Bioengineering and Materials Science & Engineering in the College of Engineering at the University of California, Berkeley, invite applications for a tenure-track or tenured position in biomaterials at the assistant, associate, or full professor level, to be held jointly in both departments, Bioengineering and Materials Science & Engineering, with an expected start date of July 1, 2013.

We seek an individual with demonstrated excellence in the broad areas of biomaterials and bionanoscience, including biocompatible, bio-hybrid and soft biological materials. Applicants must have a Ph.D. or equivalent by date of hire and evidence of outstanding scholarship within a relevant discipline. Successful applicants are expected to offer undergraduate and graduate courses in bioengineering and materials science and engineering, and have a strong commitment to, and potential for excellence in, teaching, research, and service. The level of appointment is based on experience and qualifications. The successful applicant will enjoy outstanding opportunities for collaboration with distinguished researchers in related departments and colleges at Berkeley, the University of California, San Francisco (UCSF), the Lawrence Berkeley National Laboratory (LBNL), and within the greater Bay Area and Silicon Valley biotech communities. This exceptional environment for teaching and research offers the successful candidate a unique opportunity for intellectual and technological leadership in several areas of scholarship.

Applications should be submitted online at <http://aprecruit.berkeley.edu/apply/JPF00095> and should include a cv, cover letter, statements of research and teaching interests, selected publications, and contact information for three to five references. All letters will be treated as confidential per University of California policy and California state law. Please refer potential referees, including when letters are provided via a third party (i.e., dossier service or career center), to the UC Berkeley statement of confidentiality: <http://apo.chance.berkeley.edu/evalltr.html>. While online application submission is preferred, we will also accept application materials sent by mail to the **Department of Bioengineering, 306 Stanley Hall MC 1762, UC Berkeley, Berkeley, CA 94720-1762.**

The deadline for applications is **February 1, 2013**. Candidates will be reviewed as applications are received, so early application is recommended.

The University of California is an Equal Opportunity Affirmative Action Employer, committed to excellence through diversity. The college seeks candidates whose research, teaching, or service has prepared them to contribute to our commitment to diversity and inclusion in higher education. The college is also committed to addressing the family needs of faculty, including dual career couples and single parents. For more information please go the CALcierge web site at <http://calcierge.berkeley.edu/>.



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Chair • Department of Ecology and Evolution

Stony Brook University's Department of Ecology and Evolution is seeking an individual with an outstanding academic background in any field of ecology or evolutionary biology to serve as the Department Chair. The successful candidate should have internationally recognized research credentials, a track record of extramurally funded research, demonstrated commitment to excellence in research and teaching, and proven leadership skills in an academic environment. Applicants should present a compelling vision to guiding the future trajectory of the Department and capitalizing on new initiatives put forward by the Department and University. The successful candidate will teach graduate and/or undergraduate courses according to his or her area of expertise.

Ecology and Evolution is a dynamic and growing department in an AAU university offering competitive teaching loads and start-ups. Information about Department faculty and our strong graduate training program is available at <http://life.bio.sunysb.edu/ee/>. Areas of strength in our program include population genetics, conservation ecology, molecular evolution and phylogenetics, evolutionary genomics, species interactions, invasion ecology, biogeography, mathematical ecology, and marine and freshwater ecology. The Department has recently benefited from a number of campuswide multidisciplinary initiatives that have resulted in new hires and collaborations. NYSUNY 2020 has reinvigorated Stony Brook University with increasing resources and a drive for academic excellence. The University is a member of the prestigious Association of American Universities and co-manager of nearby Brookhaven National Laboratory, a multidisciplinary research laboratory supporting world-class scientific programs utilizing state-of-the-art facilities. Stony Brook University Hospital is Suffolk County's only academic medical center and tertiary care provider. Collaborations are also possible with Cold Spring Harbor Laboratory. The campus is close to marine and terrestrial research sites, including 50,000 acres of legally protected pine barrens and woodlands. Located in the New York metropolitan area, Stony Brook is situated on the North Shore of Long Island, New York, with access to farmlands, vineyards, miles of beaches and convenient access to the cultural resources of New York City.

Applicants must hold a PhD in Ecology, Evolution, Statistics or a related field, and have demonstrated excellence in research and leadership. Applications are due January 15, 2013. Applicants should complete the Academic Jobs application process online at <https://academicjobsonline.org/ajob/jobs/2297>. The application process consists of: 1) a cover letter detailing administrative leadership experiences and philosophy, 2) a statement of research and teaching experience, 3) a curriculum vitae, and 4) the names and contact details of three academic referees. Electronic submission via [academicjobsonline](https://academicjobsonline.org/ajob/jobs/2297) is strongly preferred.

For a full position description, application procedures and to apply online, visit <https://academicjobsonline.org/ajob/jobs/2297> or visit www.stonybrook.edu/jobs (Job Reference #: F-7500-12-09). Alternatively, applicants may submit the application materials by mail to:

Chair of Search Committee, c/o Donna DiGiovanni, Assistant to Chair
Department of Ecology and Evolution, Life Sciences Building, Room 650
Stony Brook University, Stony Brook, NY 11794-5245



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DR. SHIRLEY MALCOM



To Dr. Shirley Malcom, born and raised in the segregated South more than 65 years ago, a career based on her studies in science seemed even less likely than the launch of the Soviet's Sputnik. But with Sputnik's success, the Space Race officially started and, in an instant, brought a laser-like focus to science education and ways to deliver a proper response. Not long after, Dr. Malcom entered the picture.

Although black schools at the time received fewer dollars per student and did not have sufficient resources to maintain their labs at a level equivalent to the white schools, Dr. Malcom found her way to the University of Washington where she succeeded in obtaining a B.S. in spite of the difficulties of being an African American woman in the field of science. From there she went on to earn a Ph.D. in ecology from Penn State and held a faculty position at the University of North Carolina, Wilmington.

Dr. Malcom has served at the AAAS in multiple capacities, and is presently Head of the Directorate for Education and Human Resources Programs. Nominated by President Clinton to the National Science Board, she also held a position on his Committee of Advisors on Science and Technology. She is currently a member of the Caltech Board of Trustees, a Regent of Morgan State University, and co-chair of the Gender Advisory Board of the UN Commission on Science and Technology for Development. She has held numerous other positions of distinction and is the principal author of *The Double Bind: The Price of Being a Minority Woman in Science*.

Of her active career in science, Dr. Malcom says, "I guess I have become a poster child for taking one's science background and using that in many other ways: we ask questions; we try to understand what we find; we consider what evidence we would need to confirm or refute hypotheses. And that happens in whatever setting one finds oneself."

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PRIZES

VANDERBILT UNIVERSITY  School of Medicine

CALL FOR NOMINATIONS

2013 Vanderbilt Prize in Biomedical Science

The Vanderbilt Prize in Biomedical Science was created to honor and **recognize a woman scientist of national reputation** who has a stellar record of research accomplishments and is known for her mentorship of women. The winner nurtures the career, research, and studies of a promising woman beginning her Ph.D. studies at Vanderbilt. The Vanderbilt Prize in Biomedical Science includes a \$25,000 cash award to the recipient, who will visit Vanderbilt to give a lecture and serve as a mentor to the Vanderbilt Prize Scholar.

NOMINATIONS DEADLINE IS FEBRUARY 1, 2013

Previous winners:

2012: **Dr. Joan Steitz**, Yale University
2011: **Dr. Titia de Lange**, Rockefeller University
2010: **Dr. Nancy Andrews**, Duke University
2009: **Dr. Susan Taylor**, Univ. of California, San Diego
2008: **Dr. Ann Graybiel**, MIT
2007: **Dr. Elizabeth Blackburn**, UCSF & 2009 Nobel laureate
2006: **Dr. Nancy Andreasen** of the University of Iowa

Contact Danielle Certa for a nomination form:
danielle.certa@vanderbilt.edu | (615) 936-6228

Or visit <https://medschool.vanderbilt.edu/dean/> and click **Vanderbilt Prize in Biomedical Science.**

SYMPOSIUMS

INTERNATIONAL SOCIETY FOR NEUROCHEMISTRY AMERICAN SOCIETY FOR NEUROCHEMISTRY

Satellite Symposium

UNVEILING THE SIGNIFICANCE OF LIPID SIGNALING IN
NEURODEGENERATION AND NEUROPROTECTION

April 17 - 19, 2013

Hyatt Regency Cancun, Cancun, Mexico

The purpose of this meeting is to honor and recognize the academic contributions of Dr. Nicolas Bazan to advancing lipid signaling in neuroscience and ophthalmology, as well as his dedicated service to the ASN and ISN.

In addition, this satellite meeting will integrate signaling mediated by different lipids to neurological disorders. This integrative approach will stimulate vigorous discussions on mechanisms and novel strategies for prevention and treatment of neurological diseases.

Nicolas G. Bazan,
M.D., Ph.D., Honoree
(New Orleans, LA)

ORGANIZING COMMITTEE:

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Grace Sun, Ph.D.
(Columbia, MO)

Co-Chair -
W. Gibson Wood, Ph.D.
(Minneapolis, MN)

*Abstracts are encouraged
for poster presentations.*

*Selected abstracts
will be chosen for oral
presentation.*

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RESTORATION ECOLOGIST

Assistant/Associate Professor School of the Environment Washington State University

Washington State University (WSU) is seeking a tenure-track Assistant Professor in the area of Restoration Ecology. The position is a full-time, nine-month faculty position located on the Tri-Cities campus in Richland, Washington. The position is part of a multi-year series of new hires intended to contribute to the growth and development of the new School of the Environment, which has identified the following thematic areas of focus: (1) "Water: Connecting Earth and Life," (2) "Global Change: Sustaining Healthy Landscapes and Communities," and (3) "Earth System Dynamics." Responsibilities include developing and teaching undergraduate and graduate courses and mentoring M.S. and Ph.D. graduate students. The School of the Environment has a single faculty and a common curriculum across the Pullman, Tri-Cities, and Vancouver campuses of the WSU System.

The Restoration Ecologist will develop an internationally recognized research program in analysis and restoration. Expertise in analysis at the landscape scale is particularly encouraged. Required: Earned doctorate in a relevant discipline at time of application. Record of research accomplishment as demonstrated by peer-reviewed publications and/or extramural grantsmanship. Demonstrated ability and/or potential to successfully teach and mentor students at the graduate and undergraduate levels; minimum 24 months experience. Screening begins January 7, 2013.

The following application materials are required and should be submitted through the online system to **website: <https://www.wsujobs.com>**. (**Position #116118**); a letter describing how your experience and training meet qualifications for the position, a research plan, a statement of teaching philosophy, a current curriculum vitae, and the names and contact information for three references. Only electronic application materials will be processed. *WSU is an Equal Opportunity/Affirmative Action Educator and Employer. Members of ethnic minorities, women, Vietnam era or disabled veterans, persons with a disability and/or persons age 40 and over are encouraged to apply.*

LABORATORY LECTURER

Bryn Mawr College, Department of Biology The Department of Biology at Bryn Mawr College invites applications for a full-time continuing non-tenure-track Laboratory Lecturer position to begin August 1, 2013. The position is a three-year appointment with the potential to be renewed for multiple terms. The successful candidate will teach the laboratory portions of Introductory Biology courses and supervise undergraduate instructional assistants. Additional duties include ordering materials and supplies and maintaining laboratory equipment. Commitment to curricular development and innovative approaches to teaching are critical. An advanced degree (Ph.D. preferred) in Biology and at least one year of relevant experience by the appointment start date are required. Submit a cover letter, curriculum vitae, and statement of teaching philosophy that includes examples of innovative laboratory exercises by January 18, 2013 to: **Biology Lab Lecturer Search Committee, c/o Jodi Jacoby, Department of Biology, Bryn Mawr College, 101 N. Merion Avenue, Bryn Mawr, PA 19010**. In addition, arrange to have three letters of recommendation sent to the same address. *Bryn Mawr College is an Equal Opportunity Employer; minority candidates and women are especially encouraged to apply.*

POSITIONS OPEN



FACULTY POSITION, VIROLOGY

The University of Rochester School of Medicine and Dentistry wishes to recruit a tenure-track faculty member at any level with expertise in virology. The successful candidate will be a member of the Department of Microbiology and Immunology (**website: <http://www.urmc.rochester.edu/mbi>**), and will benefit from outstanding infrastructure, state-of-the-art core facilities, a strong graduate program, and a highly collaborative, multidisciplinary research environment. The candidate's expertise will complement a number of major NIH funded center awards and contracts at the University including the Respiratory Pathogens Research Center (RPRC), the Center of Excellence in Influenza Research and Surveillance (NYICE), and a Developmental Center for AIDS Research (DCFAR).

Qualified candidates who study basic and/or translational aspects of virology are invited to apply. Candidates conducting research on respiratory viruses or on HIV/AIDS (and associated viral agents) are especially encouraged to apply, as are individuals whose work involves the application of systems approaches to research questions in virology. Applicants with a strong record of accomplishment and innovation should apply online at **website: <http://www.rochester.edu/jobopp>**, **job number 177823**. A letter of application, curriculum vitae, statement of current research interests and future plans, PDFs of two publications, and three letters of recommendation should also be sent to **e-mail: wendy_keck@urmc.rochester.edu**.

Review of applications will start January 2, 2013.

The University of Rochester is an Equal Opportunity Employer and has a strong commitment to diversity; it actively encourages applications from groups underrepresented in higher education.

MULTIPLE FACULTY POSITIONS in Limnology/Oceanography

The University of Minnesota Duluth (UMD) invites applications for multiple tenure-track, **ASSISTANT PROFESSOR** positions in the general areas of limnology and oceanography, or related field. The positions are joint research and teaching appointments, shared between Large Lakes Observatory (LLO; **website: <http://www.d.umn.edu/llo>**) and appropriate academic departments (e.g. Biology or Physics), starting August 26, 2013. Areas of interest for research include, but are not limited to: biological remote sensing, aquatic ecology, physical limnology, and atmospheric dynamics in aquatic systems. Required qualifications include a Ph.D. (or foreign degree equivalent) in limnology, oceanography, or a related field from a university with at least the equivalent of regional accreditation in the U.S. system at the time of the appointment. Evidence of potential for successful university-level teaching and externally funded research related to limnology or oceanography is also required. A complete position description and the required online application is available at **website: <https://employment.umn.edu/applicants/central?quickFind=107362>**.

Complete applications will be reviewed starting the first week of January 2013 and will be accepted until the positions are filled.

The University of Minnesota is an Equal Opportunity Educator and Employer.

UNIVERSITY OF HAWAII (UH) AT MANOA CANCER CENTER is recruiting for Tenure-Track **ASSISTANT/ASSOCIATE/FULL RESEARCHER** in the Cancer Prevention and Control Program, with **Position #88656**. Visit the Work at UH **website: <http://www.pers.hawaii.edu/wuh/>** for complete information. For inquiries, please contact **Namrata Gurung** at **e-mail: hr@cc.hawaii.edu**. *The University of Hawaii is an Equal Employment Opportunity/Affirmative Action Institution.*

POSITIONS OPEN

ASSISTANT PROFESSOR POSITION University of Wisconsin, Madison

The Department of Kinesiology at the University of Wisconsin (UW) is seeking applications for an Assistant Professor (tenure-track) position in Exercise Physiology available starting August 26, 2013. Area of research interest is open within the broad confines of biological aspects of exercise. Earned doctorate is required and postdoctoral research experience is preferred. We are seeking individuals capable of developing and maintaining a high quality, externally funded, independent research program. We are also committed to hiring an individual who can provide quality instruction in Exercise Physiology to undergraduate and graduate students. Experience working with diverse populations is a plus. Information about the department is available at **website: <http://www.education.wisc.edu/kinesiology/>**. Applicants should send a letter of application, curriculum vitae, statement of research interests, copies of up to three published articles in refereed journals, and names, mailing addresses, e-mail addresses, and telephone numbers of three references to: **Professor Bill Schrage, University of Wisconsin, Department of Kinesiology, 2000 Observatory Dr., Madison, WI 53706**. We encourage the submission of all materials electronically in PDF or Word format to **e-mail: wschrage@education.wisc.edu**. To ensure full consideration, application should be received by January 7, 2013, but applications will be accepted until the position is filled. *UW-Madison is an Equal Opportunity/Affirmative Action Employer. We promote excellence through diversity and encourage all qualified individuals to apply. Unless confidentiality is requested in writing, information regarding the applicants must be released upon request. Finalists cannot be guaranteed confidentiality.*

UNIVERSITY OF HAWAII (UH) AT MANOA CANCER CENTER is recruiting for Tenure-Track **ASSISTANT/ASSOCIATE/FULL RESEARCHER** in the Cancer Prevention and Control Program, with **Position #88660**. Visit the Work at UH **website: <http://www.pers.hawaii.edu/wuh/>** for complete information. For inquiries, please contact **Namrata Gurung** at **e-mail: hr@cc.hawaii.edu**. *The University of Hawaii is an Equal Employment Opportunity/Affirmative Action Institution.*

UNIVERSITY OF HAWAII (UH) AT MANOA CANCER CENTER is recruiting for Tenure-Track **ASSISTANT/ASSOCIATE/FULL RESEARCHER** in the Clinical and Translational Research Program, with **Position #82569**. Visit the Work at UH **website: <http://www.pers.hawaii.edu/wuh/>** for complete information. For inquiries, please contact **Namrata Gurung** at **e-mail: hr@cc.hawaii.edu**. *The University of Hawaii is an Equal Employment Opportunity/Affirmative Action Institution.*

UNIVERSITY OF HAWAII (UH) AT MANOA CANCER CENTER is recruiting for Tenure-Track **ASSISTANT/ASSOCIATE/FULL RESEARCHER** in the Cancer Biology-Natural Products Program, with **Position #82954**. Visit the Work at UH **website: <http://www.pers.hawaii.edu/wuh/>** for complete information. For inquiries, please contact **Namrata Gurung** at **e-mail: hr@cc.hawaii.edu**. *The University of Hawaii is an Equal Employment Opportunity/Affirmative Action Institution.*

UNIVERSITY OF HAWAII (UH) AT MANOA CANCER CENTER is recruiting for Tenure-Track **ASSISTANT/ASSOCIATE/FULL RESEARCHER** in the Cancer Biology Program, with **Position #86152**. Visit the Work at UH **website: <http://www.pers.hawaii.edu/wuh/>** for complete information. For inquiries, please contact **Namrata Gurung** at **e-mail: hr@cc.hawaii.edu**. *The University of Hawaii is an Equal Employment Opportunity/Affirmative Action Institution.*

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POSITIONS OPEN

MICROBIOLOGIST

Applications are invited for a tenure-track **ASSISTANT PROFESSOR** position to begin August 2013. Teaching responsibilities will include undergraduate courses in allied health microbiology and an upper division course in virology would be preferred. Establishment of a funded research program involving M.S. graduate students and undergraduates is expected. Area of research in any branch of microbiology will be considered. Postdoctoral experience preferred. Applicants should submit a letter of application, curriculum vitae, selected recent reprints, statements of research and teaching interests and have three letters of recommendation sent by January 7, 2013 to: **Dr. Elliott Blumenthal**, Department of Biology, Indiana-Purdue University Fort Wayne, 2101 E. Coliseum Boulevard, Fort Wayne, IN 46805-1499 or electronically to e-mail: blumenth@ipfw.edu. Website: <http://www.ipfw.edu/bio/>. Employment is contingent on a satisfactory background records history check. Indiana-Purdue University Fort Wayne is an Affirmative Action/Equal Access Employer fully committed to achieving a diverse workforce.

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